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

F. A. RAINNEY, S. KLATTE, R. M. KROPPENSTEDT, AND E. STACKEBRANDT*

*DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, D-38124 Braunschweig, Germany*

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* and *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 phosphatidylglycerol and phosphatidylglycerol 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.

---

**MATERIALS AND METHODS**

**Bacterial strains and cultivation.** *R. maris* DSM 43672 (T = type strain) and DSM 46102, *R. erythropolis* DSM 43066<sup>T</sup>, *Rhodococcus rhodochrous* DSM 43241<sup>T</sup>, *Gordona bronchialis* DSM 43249<sup>T</sup>, and *Mycobacterium chlorophenolicum* (formerly *Rhodococcus chlorophenolicus*) DSM 43826<sup>T</sup> were grown on trypticase soy agar (3% [wt/vol] Trypticase soy broth [BBL], 1.5% [wt/vol] Dextrose 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*, *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.

---

* Corresponding author. Mailing address: DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1B, D-38124 Braunschweig, Germany. Phone: 49 531 2616 352. Fax: 49 531 2616 418. Electronic mail address: Stackebrandt@venus.gbf-braunschweig.de@venus.gbf-braunschweig.de.

---

**RESULTS AND DISCUSSION**

The almost complete 16S rDNA sequences of *R. maris* DSM 43672<sup>T</sup> 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 paurometabolus*, as well as 10 additional species of...
Rhodococcus rhodochrous
Rhodococcus erythropolis
Rhodococcus fascians
Nocardia asteroides
Nocardia otitidiscaviarum
Gordonia bronchialis
Gordonia terrae
Mycochromobacterium chlorophenolicum
Mycochromobacterium tuberculostercum
Tsukamurella paurometabolum
Dietzia maris
Corynebacterium xerosis
Corynebacterium glutamicum
Actinomyces naesiu spp. anitratus
Propionibacterium acnes
Mycolicoccus methanolicus
Sporichthya polymorpha
Dactylosporangium thailandense
Acidothermus cellulolyticus
Bifidobacterium bifidum
Aspergillus minuta

0.10

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 rRNA 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 rRNA 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 rRNA sequences, branched off after the Corynebacterium line of descent, but before the main radiation that includes members of the genera Tsukamurella, Mycobacterium, Gordonia, and Nocardia and the authentic Rhodococcus species. The 16S rRNA 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 Gordonia species (19). The uniqueness of the 16S rRNA 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), 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 carbon atoms in the parent mycyclic 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, Gordonia, 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-acetylmycolic acid residue, while all other members of the related taxa contain the rare compound N-glycolylmycolaric 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 (Dietz'ia. M.L. dim. ending -ia; M.L. fem. n. Dietzia, honoring Alma Dietz, an American microbiologist). Gram-positive, non-spor-forming cocci that germinate into short rods. Aerobic and chemooxygenic. Catalase positive. Rod-shaped cells exhibit a morphogenetic cycle. The diagnostic amino acid of the peptidoglycan is meso-diaminopimelic acid (variation Aly); the muropeptide consists of the following sugars (U-G), (G-C), and (A-U). The 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), 837 to 849 (G-C), and 987 to 1218 (A-U).
TABLE 1. 16S rDNA sequence similarity matrix for *R. maris*, reference organisms belonging to mycolic acid-containing genera, and other actinomycetes

| Organism                      | Rhodococcus maris | Corynebacterium xerosis | "Corynebacterium polymorphus" | Mycobacterium tuberculosis | Mycobacterium chelonae | Gordona bronchialis | Gordona tenea | Tsukamurella paurometabolum | Nocardia asteroides | Rhodococcus rhodochrous | Rhodococcus erythreus | Amycolatopsis methanolica | Actinomyces naeslundii subsp. anitratus | Propionibacterium acnes | Acidothermus cellulans | Streptomyces coelicolor | Dactylosporangium thailandense | Actinoplanes minutum  |
|-------------------------------|-------------------|-------------------------|-------------------------------|---------------------------|------------------------|---------------------|--------------|--------------------------------|-------------------|---------------------------|-----------------------|-----------------------------|-----------------------------|-----------------------------|------------------------|-------------------------|-----------------------------|-----------------------------|------------------------|
| *Rhodococcus maris*           | 100               |                         |                               |                           |                        |                     |              |                                |                   |                           |                       |                             |                             |                             |                        |                        |                          |                          |                        |
| *Corynebacterium xerosis*     | 94.1              | 100                     |                               |                           |                        |                     |              |                                |                   |                           |                       |                             |                             |                             |                        |                        |                          |                          |                        |
| "Corynebacterium polymorphus" | 93.2              | 95.8                    | 100                           |                           |                        |                     |              |                                |                   |                           |                       |                             |                             |                             |                        |                        |                          |                          |                        |
| *Corynebacterium glutamicum*  | 92.3              | 94.9                    | 94.7                          | 100                       |                        |                     |              |                                |                   |                           |                       |                             |                             |                             |                        |                        |                          |                          |                        |
| *Mycobacterium tuberculosis*  | 93.2              | 93.8                    | 92.2                          | 91.2                      | 100                    |                     |              |                                |                   |                           |                       |                             |                             |                             |                        |                        |                          |                          |                        |
| *Mycobacterium chelonae*      | 95.0              | 93.7                    | 92.1                          | 91.8                      | 97.0                    | 100                 |              |                                |                   |                           |                       |                             |                             |                             |                        |                        |                          |                          |                        |
| *Gordona bronchialis*         | 93.6              | 93.1                    | 92.1                          | 91.4                      | 97.9                    | 95.0                | 100          |                                |                   |                           |                       |                             |                             |                             |                        |                        |                          |                          |                        |
| *Gordona tenea*               | 93.8              | 92.8                    | 91.9                          | 91.1                      | 94.0                    | 95.0                | 98.7         | 100                          |                   |                           |                       |                             |                             |                             |                        |                        |                          |                          |                        |
| *Tsukamurella paurometabolum* | 95.7              | 93.2                    | 92.5                          | 91.4                      | 93.9                    | 96.1                | 94.4         | 94.4                          | 100              |                           |                       |                             |                             |                             |                        |                        |                          |                          |                        |
| *Nocardia asteroides*         | 94.4              | 92.4                    | 92.4                          | 91.1                      | 94.5                    | 96.0                | 95.1         | 94.9                          | 96.0              | 100                      |                       |                             |                             |                             |                        |                        |                          |                          |                        |
| *Nocardia otitidiscaviarum*   | 94.6              | 92.7                    | 93.2                          | 91.4                      | 93.6                    | 95.6                | 95.9         | 95.5                          | 95.8              | 98.2                     | 100                  |                             |                             |                             |                        |                        |                          |                          |                        |
| *Rhodococcus rhodochrous*     | 95.4              | 93.8                    | 93.5                          | 92.4                      | 94.0                    | 95.9                | 95.6         | 95.8                          | 95.8              | 97.0                     | 100                  |                             |                             |                             |                        |                        |                          |                          |                        |
| *Rhodococcus fascians*        | 94.5              | 92.9                    | 92.9                          | 92.1                      | 93.6                    | 94.7                | 94.4         | 94.7                          | 94.9              | 95.7                     | 95.9                 | 95.8                        |                             |                             |                        |                        |                          |                          |                        |
| *Rhodococcus erythreus*       | 95.9              | 92.3                    | 93.6                          | 92.0                      | 93.6                    | 95.1                | 94.4         | 94.4                          | 95.5              | 96.6                     | 96.8                 | 96.7                        | 97.6                        |                             |                        |                        |                          |                          |                        |
| *Amycolatopsis methanolica*   | 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                      |                        |                          |                          |                        |
| *Acinomyces naeslundii subsp. anitratus* | 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 |                        |                          |                        |                          |                        |                        |
| *Propionibacterium acnes*     | 89.2              | 88.7                    | 88.9                          | 89.0                      | 89.3                    | 90.2                | 90.3         | 90.0                          | 89.6              | 89.8                     | 89.9                 | 89.3                        | 89.7                        | 88.9                        | 86.6                     | 100                      |                        |                        |                        |
| *Sporichthya polymorpha*      | 92.8              | 90.2                    | 89.5                          | 89.6                      | 89.7                    | 91.7                | 91.1         | 90.7                          | 91.8              | 92.1                     | 92.5                 | 91.3                        | 92.1                        | 92.6                        | 86.6                     | 88.0                     | 100                      |                        |                        |
| *Arthrobacter globiformis*    | 92.6              | 91.6                    | 91.4                          | 91.5                      | 90.1                    | 92.0                | 91.6         | 91.2                          | 92.0              | 91.5                     | 92.0                 | 91.2                        | 91.8                        | 90.9                        | 88.8                     | 89.9                     | 91.9                     | 100                      |                        |
| *Bifidobacterium bifidum*     | 85.9              | 86.4                    | 86.7                          | 86.8                      | 85.3                    | 85.9                | 85.9         | 85.9                          | 85.6              | 86.4                     | 87.1                 | 85.3                        | 85.7                        | 86.3                        | 84.0                     | 85.4                     | 86.2                     | 87.5                     | 100                      |
| *Acidothermus cellulans*      | 90.6              | 89.1                    | 88.9                          | 88.5                      | 89.6                    | 91.1                | 90.6         | 90.2                          | 90.7              | 91.6                     | 91.7                 | 90.7                        | 91.1                        | 90.7                        | 87.1                     | 84.4                     | 87.1                     | 92.3                     | 90.1                     | 84.7                     | 100                      |
| *Streptomyces coelicolor*      | 91.0              | 89.5                    | 88.6                          | 89.6                      | 89.7                    | 91.2                | 90.9         | 90.9                          | 91.2              | 91.8                     | 92.2                 | 91.7                        | 91.8                        | 92.5                        | 85.6                     | 88.5                     | 89.2                     | 91.4                     | 86.0                     | 89.9                     | 100                      |
| *Dactylosporangium thailandense* | 91.0 | 89.5 | 88.6 | 89.6 | 89.7 | 91.2 | 90.9 | 90.9 | 91.2 | 91.8 | 92.4 | 92.1 | 90.5 | 91.3 | 91.8 | 87.0 | 89.1 | 93.4 | 91.4 | 86.5 | 91.4 | 90.4 | 100 |
| *Actinoplanes minutum*        | 84.5              | 83.3                    | 83.3                          | 83.1                      | 82.3                    | 83.8                | 83.3         | 83.0                          | 85.0              | 84.2                     | 84.3                 | 83.7                        | 84.6                        | 84.1                        | 82.3                     | 85.9                     | 84.1                     | 81.9                     | 85.0                     | 84.6                     | 84.3                     | 100                      |
urated fatty acids. Tuberculostearic acid is present. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. Phosphatidylglycerol and phosphatidylglycerol 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 Dietzia 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, Kassunova, 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. Cocccoid 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.

Acetomethylcarbinol, 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, cellulose, galactitol, galactose, inositol, lactose, maltose, manitol, 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).

**ACKNOWLEDGMENT**

We thank Gabriele Pötter for the polar lipid analyses.

**REFERENCES**


---

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

<table>
<thead>
<tr>
<th>Genus</th>
<th>Overall no. of carbon atoms in mycolic acids</th>
<th>Fatty acid type</th>
<th>Presence of</th>
<th>Menaquinone(s)</th>
<th>N-Glycolyl in glycan moiety of wall</th>
<th>Guanine-plus-cytosine content of DNA (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietzia</td>
<td>34-38</td>
<td>1B</td>
<td>+</td>
<td>-</td>
<td>MK-8(H₂)</td>
<td>73</td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>22-36</td>
<td>1A</td>
<td>+</td>
<td>+</td>
<td>MK-8(H₂), MK-9(H₂)</td>
<td>1-67</td>
</tr>
<tr>
<td>Gordona</td>
<td>46-66</td>
<td>1B</td>
<td>+</td>
<td>+</td>
<td>MK-9(H₂)</td>
<td>63-69</td>
</tr>
<tr>
<td>Mycobacterium</td>
<td>60-90</td>
<td>1B</td>
<td>+</td>
<td>-</td>
<td>MK-9(H₂)</td>
<td>72-70</td>
</tr>
<tr>
<td>Nocardia</td>
<td>44-60</td>
<td>1B</td>
<td>-</td>
<td>+</td>
<td>MK-8(H₂)</td>
<td>64-72</td>
</tr>
<tr>
<td>Rhodococcus</td>
<td>34-64</td>
<td>1B</td>
<td>+</td>
<td>+</td>
<td>MK-8(H₂)</td>
<td>63-73</td>
</tr>
<tr>
<td>Tsukamurella</td>
<td>64-78</td>
<td>1B</td>
<td>+</td>
<td>+</td>
<td>MK-9</td>
<td>67-68</td>
</tr>
</tbody>
</table>

* Data from references 4, 7, and 19.

* Fatty acid types as defined in reference 14.

* Some Corynebacterium species have fatty acid type 1B (10, 18).

* Some Corynebacterium species contain phosphatidylethanolamine (10, 18).

* Data from reference 11.

---

**FIG. 2. Two-dimensional thin-layer chromatogram of phospholipids of R. maris DSM 43672**. 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 (80:15:2:4, 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.


