Phylogenetic Analysis of the Genera *Cellulomonas*, *Promicromonospora*, and *Jonesia* and Proposal To Exclude the Genus *Jonesia* from the Family *Cellulomonadaceae*

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The 16S rRNA gene sequences of eight *Cellulomonas* species, two *Promicromonospora* species, and *Jonesia denitrificans* were determined, and these sequences were compared with the sequences of about 50 representatives of the *Arthrobacter* line of descent in the order *Actinomycetales*. We found that in spite of its current assignment to the family *Cellulomonadaceae*, *J. denitrificans* branches outside the radiation of this taxon and cannot be considered a member of it. The two *Promicromonospora* species do not cluster separately from *Cellulomonas* species and are more closely related to *Cellulomonas* species than to each other.

The family *Cellulomonadaceae* currently contains the following four genera, which have been deemed to be phylogenetically related on the basis of partial 16S rRNA sequence data: *Cellulomonas, Oerskovia, Promicromonospora*, and *Jonesia* (18, 21, 22, 24–27). One of these genera, the genus *Jonesia*, is the most distilligenus genus in terms of chemotaxonomic properties, as exemplified by a DNA G+C content that is about 15 mol% lower than the G+C contents found in the other members of the family and by the presence of fully saturated isoprenologs (MK-9); the other members of the family possess hydroxynated menaquinones of the MK-9(H4) type (18). The placement of the genus *Jonesia* in the family *Cellulomonadaceae* was not unambiguous (26, 27) because the branching point of the only species, *Jonesia denitrificans*, did not fall consistently within the radiation of *Cellulomonas* but changed according to the number and choice of reference organisms included in analyses of 16S rRNA catalogs.

A second problem concerns the relatedness of the genera *Cellulomonas* and *Oerskovia*. Members of these two genera have been found to be phylogenetically intermixed (22), which has led to the transfer of *Oerskovia* species to the genus *Cellulomonas* (28). On the other hand, morphological characteristics and the amino acid compositions of peptidoglycans, characters which have been used in the past to delineate actinomycete genera, differ in members of these two genera (5, 20, 21, 22). A taxon name does not become invalid because of a proposed taxonomic rearrangement (13), and the genus *Oerskovia* was included as a separate genus in the description of the family *Cellulomonadaceae* (27).

To provide a phylogenetically more stable basis for the genera of the family *Cellulomonadaceae* and to identify the position of the genera mentioned above within the radiation of the *Arthrobacter* branch of the order *Actinomycetales*, we sequenced the almost complete 16S ribosomal DNAs (rDNAs) of the type strains of all validly described *Cellulomonas* species and compared these sequences with the homologous sequences available for other actinomycetes.

MATERIALS AND METHODS

The 16S rDNA gene sequences of eight *Cellulomonas* species, two *Promicromonospora* species, and *Jonesia denitrificans* were determined, and these sequences were compared with the sequences of about 50 representatives of the *Arthrobacter* line of descent in the order *Actinomycetales*. We found that in spite of its current assignment to the family *Cellulomonadaceae*, *J. denitrificans* branches outside the radiation of this taxon and cannot be considered a member of it. The two *Promicromonospora* species do not cluster separately from *Cellulomonas* species and are more closely related to *Cellulomonas* species than to each other.

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**RESULTS AND DISCUSSION**

The almost complete 16S rDNA sequences of the 13 strains which we investigated, which consisted of between 1,420 and 1,480 nucleotides, were aligned with other sequences available
for a selection of species that define the arthrobacterial lineage in the order Actinomycetales. Binary similarity values were determined for members of the genera Agromyces, Arthrobacter, Brachybacterium, Brevibacterium, Clavibacter, Curtobacterium, Dermabacter, Dermatophilus, Microbacterium, Micrococcus, Rathayibacter, Renibacterium, Rothia, and Terrabacter, and a similarity matrix was generated (Table 1). The overall level of 16S rDNA similarity for members of this lineage was high; the 16S rDNA similarity values were greater than 89% (data not shown).

**Interfamily relationships.** When the sequence of Nocardioides simplex was used as a reference to root the Arthrobacter lineage (4), distance matrix analyses of dissimilarity values revealed that the majority of the strains which we studied are members of the following four main clusters: the Arthrobacter-Micrococcus group (12), the family Microbacteriaceae (17), the Cellulomonas group, and the Brevibacterium-Brachybacterium group (Fig. 1). *Dermatophilus congolensis* branches as an individual lineage. The position of the two *Brevibacterium* species does not agree with the position described in a recent report (1) in which the authors found that members of this genus represent a distinct actinomycete line of descent that exhibits no specific affinity to the genus *Arthrobacter* and its relatives. Both of the distance matrix algorithms used in this study suggested that the genus *Brevibacterium*, together with *J. denitrificans*, *Dermabacter hominis*, and *Brachybacterium faecium*, branches within the radiation of the *Arthrobacter* lineage. Low bootstrap values, however, indicated that the proposal that the *Arthrobacter-Brevibacterium* lineage has a common origin is not supported by the results of a statistical analysis. The phylogenetic position of *J. denitrificans* outside the radiation of the family *Cellulomonadaceae* is not unexpected. The same situation was found in several analyses of tRNA catalogs which were based on a significantly smaller number of sequences (18, 26). In those cases *P. citrea* also branched outside the family (18). *J. denitrificans* has a significantly lower DNA G+C content (56 to 58 mol%) than the cellulomonads (70 to 76 mol%). However, placement of this species outside the radiation of the *Cellulomonadaceae* is probably not an artifact induced by its G+C composition because the G+C content of the rRNA gene of this species (56.6 mol%) is within the range of values found for members of the cellulomonad lineage (54.9 to 58.3 mol%). In several chemotaxonomic properties, such as the amino acid composition of the peptidoglycan, the presence of teichoic acids (6), the menaquinone type, and the G+C composition of the DNA (18), *J. denitrificans* differs from members of the genera *Cellulomonas* and *Promicromonospora*, and these properties were not given due consideration in the original discussion concerning the allocation of the genus *Jonesia* to the family *Cellulomonadaceae* (26). Now that the phylogenetic relationship between the genus *Jonesia* and the *Cellulomonas-Promicromonospora* group cannot be confirmed, the rationale for keeping the genus *Jonesia* in this family has vanished.

On the basis of 16S rDNA similarity data, the closest (albeit distant) relatives of *J. denitrificans* are *Dermabacter hominis* and *Brachybacterium faecium*. While *Dermabacter hominis*, a gram-positive, asporogenous, rod-shaped bacterium (9), has been isolated from human skin, *Brachybacterium faecium* is a pleomorphic coryneform bacterium that has been isolated from poultry deep litter (2). These two taxa are chemotaxonomically similar in that they possess meso-diaminopimelic acid in the peptidoglycan, fully unsaturated menaquinones, and fatty acid and polar lipid profiles frequently found in members of the *Arthrobacter* lineage (8). The presence of unsaturated menaquinones is shared with *J. denitrificans* but is not exclusive to members of the lineage. Interestingly, a loose relationship between *J. denitrificans* and *Brachybacterium faecium* was observed in an early numerical taxonomic analysis (7). In contrast to the previously published amino acid compositions of peptidoglycans (2, 9), neither *Dermabacter hominis* nor *Brachybacterium faecium* contains a directly cross-linked type A4γ murein, as found in other members of the genera *Brevibacterium* and *Dermatophilus*, but these organisms contain a type A4γ murein. While in *Dermabacter hominis* the peptide subunits are cross-linked by a glutamyl-asparagyl dipeptide, a diglutamyl peptide has been detected in cells of *Brachybacterium faecium*; in both taxa the α-carboxyl group of ω-glutamic acid is replaced by glycine. In a previous phylogenetic study (1), *Dermabacter hominis* occupied an individual subline of descent in the *Arthrobacter* lineage, exhibiting a branching point at the root of three members of the *Arthrobacter* group. Even though the recent increase in the number of sequences of

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**TABLE 1. 16S rDNA similarity values for the type strains of species belonging to the family Cellulomonadaceae and some reference species investigated in this study**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Cellulomonas flavogrisea</th>
<th>Cellulomonas gilula</th>
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<th>Cellulomonas biazorae</th>
<th>Cellulomonas fiml</th>
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<th>Cellulomonas cellae</th>
<th>Promicromonospora carotinophila</th>
<th>Promicromonospora citrea</th>
<th>Promicromonospora citrea</th>
<th>Terrabacter tunaensis</th>
<th><strong>Kocuria denitrificans</strong></th>
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<td>97.2</td>
<td>96.7</td>
<td>96.5</td>
<td>96.5</td>
<td>96.5</td>
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members of this lineage from about 10 to about 50 (1, 11, 12, 17) allows much more precise measurement of relationships, a specific relationship between *Dermabacter hominis* and members of the *Arthrobacter-Micrococcus* group cannot be confirmed by results of this study.

Intrafamily relationships. When measured by the levels of similarity of the 16S rDNAs of the most unrelated members, the phylogenetic depth of the family *Cellulomonadaceae* is as great as the depth that separates the five genera that belong to the *Microbacteriaceae* and the four genera in the *Arthrobacter-Micrococcus* group. In these taxa, the traditionally defined genera are not always clearly separated by a combination of phenotypic and chemotaxonomic properties, and their taxonomy is presently under revision (11, 17). Except for confirmation of the intermixing of *Cellulomonas* and *Oerskovia* species (22), the intrafamily relationships of the members of the *Cellulomonadaceae* which we observed were not expected. While in a previous phylogenetic analysis of members of the genera *Cellulomonas* and *Promicromonaspora* based on the partial 16S rRNA sequences of five species, workers found that *P. citrea* groups outside the radiation of *Cellulomonas* species, the situation is different when almost all species of the genera *Cel-
**lulomonas** and **Promicromonospora** are included. Type species *P. citra* clusters with *Cellulomonas cellulans*, while *P. enterophila* is closely related to *Cellulomonas turbata* (level of sequence similarity, 99.4%). The close relationship between the latter two species has been noted previously (27), and DNA-DNA reassociation experiments will be required to determine whether these two taxa should be combined. Differences between members of the *Cellulomonas-Oerskovia* group and *Promicromonospora* species are mainly phenotypic. Unlike *cellulomonas*, *Cellulomonas* (*Oerskovia*) *turbata* and *Promicromonospora* species do not form branched rods but produce persistent or transient branching mycelia which fragment in older cultures. *Promicromonospora* species produce aerial mycelia (which have never been detected in *Cellulomonas* [*Oerskovia*] *turbata*), form motile elements, and are strictly aerobic (15, 27).

Unexpectedly, *T. tumescens* appears to share ancestry with the *cellulomonas*. In previous reports (4, 14), workers found that this species branched more deeply and close to the root of the *Arthrobacter* lineage. The difference between these reports and our findings may be due to the lengths of the 16S rDNA sequences compared. All previous investigations were based on a data set in which the 3′-terminal 200 nucleotides of the sequence were missing, while the data sets compared in this study included about 98% of the complete sequence.

**Intrageneric structure of the genus Cellulomonas.** The relationship among certain *Cellulomonas* species determined by 16S rDNA analysis is in good agreement with the results of DNA-DNA reassociation studies (22, 23). The species pair *Cellulomonas biaziota* and *Cellulomonas fimii* and the species pair *Cellulomonas gelida* and *Cellulomonas uda* exhibit high levels of 16S rDNA similarity (99.7% and 98.8%, respectively) and have significant phylogenetic relationships, as indicated by bootstrap values of 100 and 60%, respectively, and the corresponding levels of DNA similarity are high (55 and 45%, respectively). *Cellulomonas flavigena* and *Cellulomonas cellulae* are closely related to the *Cellulomonas gelida-Cellulomonas uda* group (bootstrap value, 85%) and the *Cellulomonas biaziota-Cellulomonas fimii* group (bootstrap value, 100%), respectively. *Cellulomonas cellulans* appears to be the most divergent species in the genus, as judged by the binary levels of 16S rDNA similarity with other *Cellulomonas* species (levels of similarity, 93.8 to 95.4%) and by its phylogenetic position. Fine-tuning of intrageneric relationships is not possible on the basis of low levels of DNA-DNA similarity (i.e., levels of similarity between 20 and 35%) (22, 23), and direct correlations between DNA reassociation values and 16S rDNA similarity values cannot be made. Phylogenetic separation of *Cellulomonas cellulans* from the other *Cellulomonas* species is consistent with differences in the amino acid compositions of the peptidoglycans of these organisms. *Cellulomonas cellulans*, which encompasses strains formerly classified as *Oerskovia xanthineolytica*, *Nocardia cellulae*, *Brevibacterium fermentans*, *Corynebacterium manihot*, and *Arthrobacter luteus*, has type A 4α peptidoglycan (1-Lys-D-Ser-D-Asp), while all other *Cellulomonas* species have type A 4β peptidoglycan (1-Orn-D-Asp or 1-Orn-D-Glu). Comparative analyses of the chemotaxonomic and phenotypic properties of all members of the *Cellulomonas* group are needed for a future revision of the taxonomy of these organisms.

**REFERENCES**

5. Fiedler, F., and O. Kandler. 1973. Die *Murein*typen in der Gattung *Cellu-