Phylogeny of the Genus *Corynebacterium* Deduced from Analyses of Small-Subunit Ribosomal DNA Sequences

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We determined almost complete small-subunit ribosomal DNA sequences of 50 reference strains belonging to the genera *Corynebacterium*, *Rhodococcus*, and *Gordona* and compared these sequences with previously published sequences. Three phylogenetic methods (the neighbor-joining, maximum-likelihood, and maximum-parsimony methods), as well as a bootstrap analysis, were used to assess the robustness of each topology which we obtained. The results of comparative phylogenetic analyses confirmed that the genera *Corynebacterium*, *Dietzia*, *Gordona*, *Mycobacterium*, *Nocardia*, *Tsukamurella*, and *Turicella* form a monophyletic taxon within the phylum containing the high-G+C-content gram-positive bacteria. The genus *Corynebacterium* appeared to be a monophyletic unit whose members could be divided into four major clusters. The validity of the genus *Turicella* is doubtful since members of this genus clearly belong to the genus *Corynebacterium*. The variability of chemotaxonomic characteristics within the genus *Corynebacterium* suggests that small-subunit ribosomal DNA sequence analysis is probably the most straightforward method for confirming that a bacterium belongs to this genus.

The actinomycetes that are characterized by cell wall chemotype IV (cell walls containing *meso*-diaminopimelic acid as the diamino acid and arabinose and galactose as the major sugars [26]) and contain mycolic acids include many organisms that are pathogens for humans and animals. The results of small-subunit ribosomal DNA (rDNA) cataloging (13, 47, 48), rDNA-DNA hybridization (28), and small-subunit rDNA sequence (41) analyses support the hypothesis that there is a robust monophyletic taxon (the CMN group) that includes all of these bacteria. The chemotaxonomic profile and G+C contents of these organisms led to the description of six genera organized in the following three families (15): *Mycobacteriaceae* (containing the single genus *Mycobacterium*), *Corynebacteriaceae* (containing the single genus *Corynebacterium*), and *Nocardiaceae* (containing four genera, the genera *Nocardia*, *Rhodococcus*, *Gordona*, and *Tsukamurella*). In contrast, the results of small-subunit rDNA cataloging (13) and rDNA-DNA hybridization (28) analyses revealed only two families, the family *Mycobacteriaceae* (containing the genera *Mycobacterium*, *Nocardia*, and *Rhodococcus*) and the family *Corynebacteriaceae* (containing the single genus *Corynebacterium*). Complete rRNA descriptions are now available for the genera *Mycobacterium* (30, 40, 49), *Rhodococcus*, *Gordona*, and *Nocardia* (6, 24, 25, 31–33, 41). Recently, the genus *Dietzia* was created for the single species *Rhodococcus maris*, which is now named *Dietzia maris* (33). Despite recent taxonomic revisions of several *Rhodococcus* species (Table 1), all of the remaining *Rhodococcus* species were not grouped in a monophyletic taxon but were placed in six groups on the basis of small-subunit rDNA analysis results (31). The genera *Gordona* and *Nocardia* constituted two monophyletic taxa after exclusion of "*Nocardia amarae*," which was transferred to the genus *Gordona*, and transfer of *Rhodococcus aichiensis* to the genus *Gordona* as *Gordona aichiensis*; these transfers were supported by chemotaxonomic data (17, 25, 41). Detailed information con-

cerning phylogenetic relationships among members of the genus *Corynebacterium* is still not available, however, because small-subunit rDNA sequences have not been determined for a sufficient number of species. To help resolve phylogenetic relationships within the genus *Corynebacterium* and to examine the phylogenetic position of this genus within the CMN group, we determined nearly complete sequences of the small-subunit rDNAs of 50 reference strains belonging to the genera *Corynebacterium*, *Rhodococcus*, and *Gordona*. We combined our data with previously published data, and we discuss our results in terms of chemotaxonomy in an attempt to describe intrageneric relationships within the genus *Corynebacterium* and intergeneric relationships with other closely related taxa.

MATERIALS AND METHODS

Bacterial strains. We determined the small-subunit rDNA sequences of *Corynebacterium*, *Rhodococcus*, and *Gordona* strains whose small-subunit rDNA sequences were not available when this study was initiated; the strains which we used are listed in Table 1. Table 1 also shows the sources of strains and EMBL accession numbers. The culture medium and culture conditions used were the medium and conditions recommended in the *American Type Culture Collection Catalogue of Bacteria and Bacteriophages* (1).

PCR amplification and small-subunit rDNA sequencing. The method used to prepare bacterial DNA for PCR was derived from the method of Sritharan and Barker (46). Colonies were suspended in 200 μ l of a lysis solution (10 mM Tris-HCl, 1 mM EDTA, 1% Triton X-100; pH 8), heated for 5 min at 100°C, and then transferred on ice. After a single chloroform extraction, 5 μ l of supernatant was used in a PCR to amplify the small-subunit rDNA genes. Two pairs of primers were used; the members of one pair corresponded to positions 8 to 28 and 1498 to 1509 on the *Escherichia coli* small-subunit rDNA sequence, and the members of the other pair corresponded to positions 8 to 28 and 1384 to 1400. The procedures used for PCR amplification and sequencing of PCR products have been described previously (42).

Phylogenetic analysis and alignment: general procedure. The phylogenetic data described below were obtained (i) by using successive alignment and phylogeny procedures and (ii) by reinvestigating deep branching patterns after close relationships were determined. In each phylogenetic analysis, we restricted the comparisons to nucleotide positions that were aligned without doubt. Some analyses were performed several times, with or without small domains that could have reached the point of saturation for mutations. Although this approach was probably not as efficient as carefully weighting each position independently, it was easier and was probably a reasonable compromise considering the possible

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TABLE 1. Sources and EMBL accession numbers for small-subunit rDNA sequences of the bacteria sequenced in this study

Taxon ^a	Source ^b	Strain	ATCC no.	EMBL ^c	Reference(s) ^d
<i>Corynebacterium afermentans</i> subsp. <i>afermentans</i>	CIP	CIP 103499 ^T		X82054	
<i>Corynebacterium afermentans</i> subsp. <i>liphophilum</i>	CIP	CIP 103500 ^T		X82055	
<i>Corynebacterium ammoniagenes</i>	CIP	CIP 101283 ^T	ATCC 6871 ^T	X82056	
<i>Corynebacterium amycolatum</i>	CIP	CIP 103452 ^T	ATCC 49368 ^T	X82057	
" <i>Corynebacterium asperum</i> "	CIP	CIP 52.13		X82050	
<i>Corynebacterium bovis</i>	NCTC	NCTC 3224 ^T	ATCC 7715 ^T	X82051	
" <i>Corynebacterium hoagii</i> "	ATCC		ATCC 7005 ^T	X82052	
<i>Corynebacterium callunae</i>	CCUG	CCUG 28793	ATCC 15991 ^T	X82053	
<i>Corynebacterium cystitidis</i>	CIP	CIP 103424 ^T	ATCC 29593 ^T	X82058	
<i>Corynebacterium diphtheriae</i>	CIP	CIP 100721 ^T	ATCC 27010 ^T	X82059	
<i>Corynebacterium flavescens</i>	CIP	CIP 69.5 ^T	ATCC 10340 ^T	X82060	
<i>Corynebacterium glutamicum</i>	IAM	IAM 12435 ^T	ATCC 13032 ^T	X82061	
<i>Corynebacterium jeikeium</i>	CIP	CIP 103337 ^T	ATCC 43734 ^T	X82062	
<i>Corynebacterium kutscheri</i>	CIP	CIP 103423 ^T	ATCC 15677 ^T	X82063	
<i>Corynebacterium matruchotii</i>	CIP	CIP 81.82 ^T	ATCC 14266 ^T	X82065	
<i>Corynebacterium minutissimum</i>	NCTC	NCTC 10288 ^T	ATCC 23348 ^T	X82064	
<i>Corynebacterium mycetoides</i>	CIP	CIP 55.51 ^T	ATCC 21134 ^T	X82066	
<i>Corynebacterium pilosum</i>	ATCC		ATCC 29592 ^T	X81908	
<i>Corynebacterium propinquum</i>	CIP	CIP 103792 ^T		X81917	
<i>Corynebacterium pseudodiphtheriticum</i>	CIP	CIP 103420 ^T	ATCC 10700 ^T	X81918	
<i>Corynebacterium pseudotuberculosis</i> biovar <i>ovis</i>	CIP	CIP 102968 ^T	ATCC 19410 ^T	X81916	
<i>Corynebacterium pseudotuberculosis</i> biovar <i>equi</i>	CIP	CIP 52.97		X81907	
<i>Corynebacterium renale</i>	CIP	CIP 103421 ^T	ATCC 19412 ^T	X81909	
<i>Corynebacterium</i> sp. CDC group F1 taxon 1	CDC	CDC G4330		X81905	
<i>Corynebacterium</i> sp. CDC group F1 taxon 2	CDC	CDC G5911		X81904	
<i>Corynebacterium striatum</i>	CIP	CIP 81.15 ^T	ATCC 6940 ^T	X81910	
<i>Corynebacterium ulcerans</i> 1	CIP	CIP 54.53		X81912	
<i>Corynebacterium ulcerans</i> 2	CCUG	CCUG 2708 ^T	ATCC 51799 ^T	X81911	
<i>Corynebacterium urealyticum</i>	ATCC		ATCC 43042 ^T	X81913	
<i>Corynebacterium xerosis</i> 1	ATCC		ATCC 373 ^T	X81914	
<i>Corynebacterium xerosis</i> 2	IAM	IAM 12431	ATCC 7711	X81906	
<i>Dietzia maris</i> (formerly <i>Rhodococcus maris</i>)	ATCC		ATCC 35013 ^T	X81920	33
<i>Gordona aichiensis</i> (formerly <i>Rhodococcus aichiensis</i>)	ATCC		ATCC 33611 ^T	X81925	25
<i>Gordona bronchialis</i>	CIP	CIP 1780.88 ^T	ATCC 25592 ^T	X81919	
<i>Gordona rubropertinctus</i>	ATCC		ATCC 25593 ^T	X81915	
<i>Gordona sputi</i> 1	CIP	CIP 1833.89 ^T	ATCC 29627 ^T	X81923	
<i>Gordona sputi</i> 2	CIP	CIP 1835.89		X81924	
<i>Gordona sputi</i> 3 (formerly <i>Rhodococcus chubuensis</i>)	CIP	CIP 1781.88	ATCC 33609	X81927	36
<i>Gordona terrae</i>	ATCC		ATCC 25594 ^T	X81922	
<i>Mycobacterium chlorophenolicum</i> (formerly <i>Rhodococcus chlorophenolicus</i>)	NCIMB	NCIMB 12325 ^T		X81926	4, 20
<i>Rhodococcus coprophilus</i>	ATCC		ATCC 29080 ^T	X81928	
<i>Rhodococcus erythropolis</i>	ATCC		ATCC 4277 ^T	X81929	
<i>Rhodococcus fascians</i>	ATCC		ATCC 12974 ^T	X81930	
<i>Rhodococcus fascians</i> (formerly <i>Rhodococcus luteus</i>)	ATCC		ATCC 35014	X81932	24
<i>Rhodococcus globerulus</i>	NCIMB	NCIMB 12315 ^T		X81931	
<i>Rhodococcus marinonascens</i>	ATCC		ATCC 35653 ^T	X81933	
<i>Rhodococcus rhodnii</i>	ATCC		ATCC 35071 ^T	X81935	
<i>Rhodococcus rhodochrous</i> 1	CIP	CIP 1759.88 ^T	ATCC 13808 ^T	X81936	
<i>Rhodococcus rhodochrous</i> 2 (formerly <i>Rhodococcus roseus</i>)	ATCC		ATCC 271	X81921	32
<i>Rhodococcus zopfii</i>	ATCC		ATCC 51349 ^T	X81934	

^a Quotations marks indicate that an organism has been reassigned to a different genus or species.^b Collections from which the bacteria were obtained. ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection of the University of Göteborg, Göteborg, Sweden; CDC, Centers for Disease Control and Prevention, Atlanta, Ga.; CIP, Collection Institut Pasteur, Paris, France; IAM, Institute of Applied Microbiology, Tokyo, Japan; NCTC, National Collection of Type Cultures, London, United Kingdom; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, United Kingdom.^c EMBL accession numbers for small-subunit rDNA sequences.^d Papers published while this work was in progress, in which sequences are described and phylogenetic positions are analyzed.

problems of crossing-over that might affect some of the sequences investigated (45).

We also performed some analyses several times by including and excluding particular species that had high rates of mutation or that were distant outgroups. Inclusion of distantly related outgroups brings random noise into an analysis and does not allow resolution of the relationships among the more closely related species (44). Long branches for ingroups have the same effect as distant outgroups (that is, they bring noise into a phylogenetic analysis). Thus, monophyletic units containing such species are unstable because of highly variable sequences, and their presence can also affect neighboring taxa. To alleviate this problem, we performed phylogenetic analyses successively with all outgroups or with a set of outgroups restricted to the closest relatives, with or without ingroups that were characterized by long branches.

Because we wanted to keep computation time for each phylogenetic analysis within reasonable limits, it was not possible to include all representatives of the outgroups and ingroups in maximum-parsimony and maximum-likelihood analyses. Instead, we performed multiple analyses by using different outgroups and different ingroups (as determined by the neighbor-joining analysis). All sequence alignment and species selection operations were done by using computer programs developed by us and available on request from R. Christen.

Phylogenetic methods. A neighbor-joining method (43) was used in our preliminary analysis; the resulting topologies were then further investigated by using maximum-likelihood and maximum-parsimony methods. For the maximum-likelihood analyses we used the *fdnaml* program rewritten by G. J. Olsen (University of Illinois, Urbana) and compiled on a Hewlett-Packard model 700 workstation, while for the maximum-parsimony analyses we used the "PAUP" program for the Macintosh (51). The robustness of each topology was evaluated with a maximum-parsimony method through 100 bootstrap replications (heuristic search). Trees were constructed by using the *njplot* program for the Macintosh developed by M. Gouy (Université Claude Bernard, Lyon, France), which allows transformation of a formal tree representation (Newick's format) into MacDraw drawings.

Only topologies that were found to be similar by all three methods were retained as true trees. The results of recent theoretical work have demonstrated that convergence of the results of all three methods is a very robust indication that the correct phylogeny has been determined (21, 23).

Domains used. Different parts of the small-subunit rDNA sequences were used to prepare Fig. 1 and 2 and Table 2. The positions used for Fig. 1 correspond to positions 6 to 42, 91 to 145, 193 to 412, 441 to 558, 565 to 595, 600 to 782, 801 to 949, 984 to 1082, 1088 to 1206, 1214 to 1234, and 1244 to 1320 in the sequence of *Corynebacterium amycolatum*. The positions used for Fig. 2 correspond to *C. amycolatum* positions 201 to 272, 276 to 348, 350 to 402, 441 to 557, 569 to 595, 598 to 782, 802 to 900, 921 to 949, 984 to 1080, 1092 to 1201, 1214 to 1232, and 1244 to 1310. And the positions used for Table 2 correspond to *C. amycolatum* positions 2 to 48, 60 to 156, 174 to 422, and 428 to 1320.

Nucleotide sequence accession numbers. The EMBL accession number for the nucleotide sequence of *C. amycolatum* is X82057. Other EMBL accession numbers are shown in Table 1.

RESULTS AND DISCUSSION

Phylogenetic relationships within the genus *Corynebacterium*. Relationships within the genus *Corynebacterium* were studied in our phylogenetic analyses by using *Tsukamurella paurometabolum* and *Dietzia maris* as the outgroups closest to this genus. The results of these analyses demonstrated that there are four major clades in this genus (clusters I through IV) (Fig. 1).

Cluster IV contained *C. amycolatum*, "*Corynebacterium asperum*," and *Corynebacterium xerosis* ATCC 373^T (T = type strain) in a robust clade (bootstrap value, 99%) that was a sister group to all other corynebacteria (including *Turicella otitidis*) (Fig. 1). Because they exhibit a level of DNA-DNA relatedness that is greater than 90% (37), "*C. asperum*" and *C. amycolatum* are subjective synonyms. In contrast, *C. xerosis* ATCC 373^T contains mycolic acids and is thus strikingly different chemotaxonically from *C. amycolatum* and "*C. asperum*," which lack mycolic acids (3, 7).

Cluster III was identified as a branch that contained *Corynebacterium bovis*, *Corynebacterium jeikeium*, *Corynebacterium urealyticum*, and *Corynebacterium variabilis*. The robustness of this clade is doubtful as it was identified only by the neighbor-joining and maximum-likelihood methods and was not supported by bootstrap data. However, these four species all have high G+C contents, which range from 61 mol% (*C. jeikeium*)

FIG. 1. Phylogenetic relationships among *Corynebacterium* species: unrooted tree obtained by using a neighbor-joining method. The double asterisks indicate branches that were also identified by a maximum-likelihood method ($P < 0.01$). The values (expressed as percentages) above the lines (only values greater than 50% are shown) mark branches also found in the most-parsimonious tree and indicate how these branches are supported by bootstrap data (heuristic search). The number in parentheses indicates the percentage of bootstrap replications when *Turicella otitidis* was omitted from the analysis. Bar = number of accumulated changes per nucleotide. The names of organisms sequenced in this study are in boldface type.

to 74 mol% (*C. bovis*), and all except *C. jeikeium* have tuberculostearic acid in their cell walls. *C. jeikeium* and *C. urealyticum*, which are closely related as determined by all three phylogenetic methods (a conclusion supported by only moderate bootstrap values) have the same respiratory type (strictly aerobic) and are resistant to the same antibiotics.

Turicella otitidis appeared to be an isolated species that we placed in a separate cluster (cluster II) (see below).

All of the remaining *Corynebacterium* species formed a monophyletic unit (cluster I) that was supported by the results of all three phylogenetic methods and a moderate bootstrap value (40%). The bootstrap value was 80% when *Turicella otitidis* was excluded from the analyses (data not shown). The peculiar rate of mutation of *Turicella otitidis* (14) is probably responsible for the unstableness of this cluster. Within this monophyletic unit, six taxa were identified by all three phylogenetic methods and by high bootstrap values. *Corynebacterium cystitidis* and *Corynebacterium pilosum*, which are pathogens of cows and previously were known as biotypes of *Corynebacterium renale*, were found to be closely related by all three phylogenetic methods, which was consistent with the DNA-DNA relatedness values (60 to 63%) (53). Two recently characterized subspecies of *Corynebacterium afermentans* rep-

FIG. 2. Phylogenetic position of the genus *Corynebacterium* within the CMN group. For an explanation see the legend to Fig. 1. The branch robustness is not indicated for the outgroups. The genus *Corynebacterium* and *Turicella otitidis* form a well-defined and robust monophyletic unit related to *D. maris*.

representing Centers for Disease Control (CDC) coryneform absolute nonfermenter group 1 (35), *Corynebacterium mycetoides*, and two *Corynebacterium* sp. strains (CDC group F1 strains G4330 and G5911), formed a monophyletic unit that was supported by moderate bootstrap values but was consistent with DNA-DNA hybridization data which revealed that *C. afermentans* subsp. *afermentans* and *C. afermentans* subsp. *lipophilum* were more closely related to *C. mycetoides* (levels of DNA relatedness, 24 and 40%, respectively) than to any other *Corynebacterium* spp., while *C. afermentans* subsp. *afermentans* and *Corynebacterium* sp. CDC group F1 strain G5911 exhibited a DNA relatedness value of 21% (35). A monophyletic unit composed of *Corynebacterium kutscheri*, *Corynebacterium diphtheriae*, two strains of *Corynebacterium pseudotuberculosis*, and two strains of *Corynebacterium ulcerans* was supported by a high bootstrap value (91%), which confirmed numerical taxonomy data (22). Because of the presence of rhamnose in its cell wall and its low G+C content (46 to 46.2 mol%) (12, 29), the position of *C. kutscheri* in the genus *Corynebacterium* has been doubted for a long time. The results of our rDNA phylogenetic analyses clearly confirmed that this organism should be included in the genus *Corynebacterium* and were consistent with data which showed that rhamnose is not present in the cell wall of *C. kutscheri* ATCC 15677^T (5). A close relationship (but not species identity) between the two strains of *C. pseudotuberculosis* and *C. ulcerans* confirmed DNA-DNA relatedness

data (39) and was consistent with the finding that the phenotypes of these organisms differ only in the production of acid from glycogen. The presence of *C. diphtheriae* in this cluster is not surprising because *C. diphtheriae*, *C. pseudotuberculosis*, and *C. ulcerans* all have the following characteristics that distinguish them from all other members of the genus: (i) the ability to produce diphtheria toxin if they are lysogenized by a suitable bacteriophage (19), (ii) a common fatty acid pattern (9), and (iii) the ability to produce neuraminidase but not pyrazinamidase (2). *Corynebacterium propinquum*, which was recently proposed as the species name for CDC coryneform absolute nonfermenter group 3 (34), and *Corynebacterium pseudodiphtheriticum*, which differs phenotypically from *C. propinquum* only by a positive urease test, formed a tight cluster supported by bootstrap analysis data at a confidence level of 100% (the rDNA sequences exhibited a level of similarity of 99.4%). This result is consistent with the level of DNA relatedness between these two species (25%) (34). The existence of a monophyletic unit containing *Corynebacterium accolens*, *Corynebacterium macginleyi*, and *Corynebacterium* sp. CDC group G-2 strain G5840 has recently been confirmed by DNA-DNA hybridization and small-subunit rDNA sequence data (38). In this study, 45 of 51 human lipophilic diphtheroids were divided into five genomic groups, which in our study were represented by *Corynebacterium* sp. CDC group G-2 strain G5840, *C. accolens*, *C. macginleyi*, *Corynebacterium* sp. CDC group F1 strain G4330, and *Corynebacterium* sp. CDC group F1 strain G5911. A close relationship (but not species identity) between the last two strains, which exhibited a level of DNA relatedness of 35%, was supported by our data. *Corynebacterium minutissimum*, *Corynebacterium striatum*, and *C. xerosis* IAM 12431 also formed a monophyletic unit (bootstrap value, 76%). The exclusion from this cluster of *C. xerosis* ATCC 373^T (closely related to *C. amycolatum*) was consistent with the heterogeneity observed with the *C. xerosis* strains available. Whole-cell fatty acids data, DNA hybridization data (qualitative data obtained by dot blot analysis), and biochemical reaction data (11) showed that 10 strains of *C. xerosis* could be separated into six groups. *C. xerosis* IAM 12431 and ATCC 373^T belonged to two distinct groups, whereas three other strains of *C. xerosis* were indistinguishable from *C. striatum*. Since the small-subunit rDNA sequence of *C. xerosis* IAM 12431 differed by only three nucleotides from the sequence of *C. striatum*, *C. xerosis* IAM 12431 could belong to *C. striatum*. However, quantitative DNA hybridization between these two strains will be necessary to confirm this possibility. *C. minutissimum* and *C. striatum* are part of the normal flora of the human skin and mucosa. These organisms have the same cellular fatty acids, but they differ phenotypically by the presence of nitrate reductase and acid formation from maltose and they have different rDNA sequences (Table 2). A close relationship (but not species identity) between *Corynebacterium callunae* and *Corynebacterium glutamicum* was expected because of the level of genomic DNA relatedness of these organisms (37%) (27).

Phylogenetic position of the genus *Corynebacterium* within the CMN group. The genera *Corynebacterium*, *Gordona*, *Rhodococcus*, *Dietzia*, *Nocardia*, *Mycobacterium*, *Tsukamurella*, and *Turicella* formed a monophyletic taxon (the CMN group) within the high-G+C-content gram-positive phylum independently of the species selected as outgroups or ingroups and of the phylogenetic methods used, confirming previous findings (10, 13, 18, 28, 41, 47, 48). All species except *Turicella otitidis* and *C. amycolatum* have cell wall chemotype IV and contain mycolic acids. Therefore, we studied the position of the genus *Corynebacterium* in this group by using the closest outgroups

TABLE 2. Similarity matrix based on small-subunit rDNA sequences

Taxon		% Similarity of small-subunit rDNA sequences for domains including all nucleotides aligned without any ambiguity																																				
<i>Corynebacterium cystitidis</i>	100.0																																					
<i>Corynebacterium pilosum</i>	95.9																																					
<i>Corynebacterium mycetoides</i>	95.2	95.9																																				
<i>Corynebacterium afimentians</i>	95.3	95.4	97.4																																			
subsp. <i>lipophilum</i>	95.3	95.3	97.3	99.8																																		
<i>Corynebacterium afimentians</i>	94.9	95.4	97.1	97.2	97.3																																	
subsp. <i>afimentians</i>	94.9	95.6	97.0	97.0	97.0	99.6																																
<i>Corynebacterium</i> sp. CDC group F1 strain G4330	94.9	95.6	97.0	97.0	97.0	99.6																																
group F1 strain G5911	94.3	95.9	95.6	95.6	95.6	95.9	95.9																															
<i>Corynebacterium renale</i>	93.5	95.0	94.5	95.4	95.4	95.4	95.0	95.7																														
<i>Corynebacterium kutscheri</i>	93.7	95.7	95.2	95.8	95.7	95.7	95.4	95.8	97.5																													
<i>Corynebacterium diptheriae</i>	93.3	95.0	95.2	95.8	95.7	95.2	95.0	95.9	96.8	98.8																												
<i>Corynebacterium pseudotuberculosis</i> 1	93.4	95.1	95.3	95.7	95.6	95.3	95.0	96.0	96.8	98.9	99.9																											
<i>Corynebacterium pseudotuberculosis</i> 2	93.4	95.3	95.4	95.9	95.8	95.3	95.1	96.1	97.0	98.9	99.8	99.8																										
<i>Corynebacterium ulcerans</i> 1	93.4	94.7	94.9	95.6	95.5	94.7	94.6	95.6	96.7	98.3	99.2	99.3	99.4																									
<i>Corynebacterium ulcerans</i> 2	92.7	93.9	93.0	94.2	94.1	92.8	92.7	93.4	93.8	93.9	94.0	93.9	93.9	94.1																								
<i>Corynebacterium matruchotii</i>	94.7	96.0	95.7	95.8	95.7	94.6	94.8	95.5	94.4	95.5	96.2	96.1	96.3	96.3	94.6																							
<i>Corynebacterium minutissimum</i>	94.3	95.5	94.8	95.4	95.3	94.3	94.4	94.6	95.0	96.0	96.4	96.3	96.5	96.7	94.6	97.8																						
<i>Corynebacterium striatum</i>	94.3	95.5	94.8	95.4	95.3	94.3	94.4	94.6	95.0	96.0	96.4	96.3	96.7	96.5	94.6	97.8	100.0																					
<i>Corynebacterium xerosis</i> 2	93.5	94.5	94.8	95.3	94.3	94.4	94.6	94.0	93.2	94.1	94.2	94.1	94.2	94.2	93.2	95.4	94.1	94.1																				
<i>Corynebacterium pseudodiphtheriticum</i>	93.3	94.5	94.6	94.8	94.7	94.1	94.3	94.2	93.0	94.0	94.3	94.2	94.3	94.3	93.3	95.7	94.2	94.2	99.7																			
<i>Corynebacterium propinquum</i>	95.9	95.5	95.8	96.1	96.0	95.3	95.3	95.6	95.1	95.7	95.5	95.4	95.6	95.6	94.4	97.7	96.5	96.5	95.4	95.6	95.4																	
<i>Corynebacterium accolens</i>	95.9	95.3	95.7	96.0	95.9	95.0	95.1	95.3	94.7	95.3	95.2	95.1	95.3	95.2	94.3	97.3	96.0	96.0	95.2	95.4	99.4																	
<i>Corynebacterium</i> sp. strain CIP 104099	95.7	96.1	95.3	95.6	95.6	94.3	94.3	95.9	95.3	95.9	95.5	95.4	95.6	95.6	94.2	96.8	96.4	96.4	95.1	95.3	98.4	97.9																
<i>Corynebacterium</i> sp. CDC group G2 strain 5840	93.6	95.8	93.7	93.8	83.7	93.7	93.8	94.3	94.9	95.3	94.8	94.7	94.7	95.0	92.9	95.2	95.8	95.8	93.5	93.4	94.7	94.2	95.2															
<i>Corynebacterium ammoniogenes</i>	94.7	96.5	95.4	95.2	95.1	95.7	95.8	95.5	95.8	96.5	96.1	96.0	96.1	96.1	93.9	96.7	97.0	97.0	94.9	94.8	96.5	96.1	96.2	97.2														
<i>Corynebacterium flavescens</i>	93.5	94.5	93.2	93.9	93.9	93.9	93.8	93.5	93.6	94.5	94.6	94.5	94.3	94.8	92.0	94.2	94.9	94.9	92.4	92.5	93.8	93.4	93.6	95.5	95.3													
<i>Corynebacterium callunae</i>	93.7	95.2	94.0	94.7	94.7	94.6	94.6	94.3	94.5	95.3	95.2	95.1	95.1	95.1	92.6	94.8	95.0	95.0	93.2	93.3	94.7	94.3	94.6	95.2	95.7	98.2												
<i>Corynebacterium glutamicum</i>	92.6	93.8	92.6	92.6	92.5	91.8	91.6	93.1	92.1	92.0	92.7	92.7	92.7	92.6	92.0	92.9	92.6	92.6	92.3	92.3	92.4	92.6	93.0	92.6	93.4	91.6	92.0											
<i>Turicella otitidis</i>	93.0	94.3	93.9	94.4	94.3	93.6	93.7	94.0	94.4	95.5	95.8	95.7	95.8	95.8	93.0	94.6	94.9	94.9	93.9	93.6	94.0	94.2	94.7	95.1	94.5	95.3	92.3											
<i>Corynebacterium bovis</i>	93.8	94.9	94.3	94.9	94.9	94.1	93.9	94.3	93.9	95.0	94.7	94.6	94.8	95.0	93.5	95.8	94.6	94.5	94.5	95.7	95.9	95.0	94.6	95.3	94.7	95.0	92.5	96.1										
<i>Corynebacterium jeikeium</i>	93.1	94.8	94.2	94.3	94.2	93.9	93.8	95.4	94.5	95.0	95.0	95.0	95.1	95.1	93.0	95.3	94.1	94.1	94.2	94.2	95.4	95.3	95.0	94.2	95.0	93.9	94.3	92.6	95.8	97.6								
<i>Corynebacterium urealyticum</i>	93.1	94.7	94.3	94.6	94.6	94.7	94.9	94.8	94.0	95.5	95.6	95.6	95.6	95.6	93.0	95.6	95.7	95.7	93.8	93.9	95.0	94.8	94.7	95.1	96.2	95.8	93.4	96.2	96.3	95.6								
<i>Corynebacterium variabilis</i>	93.2	94.4	93.9	94.3	94.3	94.3	94.2	94.4	94.5	94.5	94.5	94.4	94.6	94.5	92.1	93.9	93.6	93.6	93.8	93.6	93.9	93.8	94.2	94.5	95.0	94.4	94.2	93.1	94.6	95.1	94.8	95.3						
<i>Corynebacterium amycolatum</i>	93.1	94.1	93.5	94.2	94.2	94.1	94.0	94.0	94.4	94.2	94.3	94.2	94.3	94.6	92.1	93.6	93.5	93.5	93.3	93.5	93.5	93.9	94.6	94.7	94.4	93.9	92.6	94.3	95.0	94.4	95.1	99.5						
" <i>Corynebacterium asperum</i> "	92.8	94.3	93.0	93.4	93.4	93.0	93.3	94.1	93.6	94.2	94.3	94.2	94.3	94.8	92.0	94.8	94.5	94.5	93.2	93.1	93.6	93.5	94.4	95.4	95.0	95.0	94.2	92.7	94.7	94.9	94.4	95.1	99.5					
<i>Corynebacterium xerosis</i> 1	91.3	92.3	92.2	92.6	92.6	92.4	92.4	92.1	92.2	92.8	92.6	92.9	92.5	92.6	91.6	92.8	93.1	93.1	92.9	92.8	92.4	92.2	92.5	92.7	93.3	92.9	92.4	90.8	93.7	94.1	93.1	93.3	94.0	94.3	94.4	95.6	97.2	97.6
<i>Rhodococcus maurus</i>	91.1	91.2	91.1	91.5	91.5	91.5	91.8	91.7	91.8	91.9	92.2	92.1	92.2	92.1	92.6	90.2	91.2	91.3	91.3	91.1	91.0	91.1	90.9	91.5	91.5	91.9	91.4	89.6	92.0	92.7	91.9	92.1	92.9	93.2	92.8	94.5	100.0	
<i>Tsukamurella paurometabolum</i>																																						
<i>Turicella otitidis</i>																																						
<i>Corynebacterium urealyticum</i>																																						
<i>Corynebacterium jeikeium</i>																																						
<i>Corynebacterium xerosis</i>																																						
<i>Corynebacterium amycolatum</i>																																						
<i>Corynebacterium variabilis</i>																																						
<i>Corynebacterium glutamicum</i>																																						
<i>Corynebacterium callunae</i>																																						
<i>Corynebacterium flavescens</i>																																						
<i>Corynebacterium aminogenes</i>																																						
<i>Corynebacterium</i> sp. CDC group G2 strain 5840																																						
<i>Corynebacterium</i> sp. strain CIP 104099																																						
<i>Corynebacterium accolens</i>																																						
<i>Corynebacterium propinquum</i>																																						
<i>Corynebacterium pseudodiphtheriticum</i>																																						
<i>Corynebacterium striatum</i>																																						
<i>Corynebacterium xerosis</i> 2																																						
<i>Corynebacterium pseudotuberculosis</i> 1																																						
<i>Corynebacterium pseudotuberculosis</i> 2																																						
<i>Corynebacterium ulcerans</i> 1																																						
<i>Corynebacterium ulcerans</i> 2																																						
<i>Corynebacterium matruchotii</i>																																						

(41, 52) to root the CMN group (Fig. 2). The genera *Gordona*, *Rhodococcus*, and *Nocardia* formed a well-defined clade with *Rhodococcus* species as deep branches. This result confirmed previously published data (31) and suggests that phenotypic traits used to define the genus *Rhodococcus* might be ancestral characters for these three genera. Numerical taxonomy, chemotaxonomy (16), DNA-DNA hybridization (50), and rDNA sequence data confirmed that "*Corynebacterium hoagii*" is a synonym of *Rhodococcus equi*. Despite the heterogeneity observed in the genus *Rhodococcus*, all of the true *Nocardia* species formed a monophyletic unit supported by the results of all phylogenetic methods and by high bootstrap values, in agreement with the results of recent detailed phylogenetic studies (6, 31, 41) and chemotaxonomic data (15). All *Gordona* species also grouped in a robust cluster supported by the results of all phylogenetic methods and by high bootstrap values. The level of rDNA similarity between *G. aichiensis* (25) and *Gordona sputi* is more than 99%, but there is less than 20% genomic DNA-DNA relatedness as determined by an S1 nuclease procedure (37).

All *Corynebacterium* species plus *Turicella otitidis* formed a robust monophyletic taxon that was a sister group of the cluster described previously (Fig. 2). The following three taxa were identified because they were supported by the results of all phylogenetic methods and by high bootstrap values: (i) *Tsukamurella paurometabolum*, (ii) *D. maris*, and (iii) the genus *Corynebacterium* (including *Turicella otitidis*). *Turicella otitidis* was clearly included in the monophyletic unit that comprised all of *Corynebacterium* species and never appeared as a deep branch within this genus. This species was elevated to the genus level on the basis of two chemical characteristics (a lack of mycolic acids and the presence of MK-10 and MK-11 as the predominant menaquinones) and by using a phylogeny based on the results of a single neighbor-joining analysis that included only three *Corynebacterium* type species (14). This classification was not confirmed by the results of our analyses, which involved three phylogenetic methods, a bootstrap analysis, and all of the *Corynebacterium* type species. Because *Turicella otitidis* exhibited an extremely broad range of dissimilarity values (Table 2) compared with all other bacteria, it is likely that the phylogenetic position of *Turicella otitidis* is easily perturbed in a neighbor-joining analysis by the well-known phenomenon of long-branch attraction. In fact, the predominant type of menaquinone remains the single chemical characteristic that distinguishes *Turicella otitidis* from other *Corynebacterium* species, as *C. amycolatum* (a true *Corynebacterium* species) also lacks mycolic acids (7). Because the menaquinone compositions of all *Corynebacterium* species have not been determined, the validity of the separate genus *Turicella* is doubtful; however, it would be prudent to obtain data for all species before the taxonomic status of *Turicella otitidis* is revised.

In conclusion, the results of our analyses of rDNA sequences confirmed that several species that do not possess the characteristic features of the genus *Corynebacterium* as outlined by Collins and Cummins (8) should be included in this genus. The initial range of G+C contents established for this genus (51 to 65 mol%) should therefore be extended to 46 to 74 mol% (*C. mycetoides*, 68 mol%; *C. afermentans*, 66 to 68 mol%; *C. bovis*, 74 mol%; *C. variabilis*, 65 mol%; *C. xerosis*, 71 mol%; and *C. kutscheri*, 46 mol%). Because *C. amycolatum* and possibly *Turicella otitidis* are included in the genus *Corynebacterium*, the presence of mycolic acids in the cell wall is not a requirement for members of the genus *Corynebacterium*. Moreover, the genus *Corynebacterium* is the only genus in the CMN group that is not characterized by the presence of a unique major

menaquinone and fatty acid type (8). The heterogeneity of these chemical characteristics probably explains why it is difficult to identify newly isolated bacteria as members of this genus. Because the genus *Corynebacterium* forms a clear monophyletic unit, small-subunit rDNA sequence analysis is probably the most straightforward method for confirming that a bacterium belongs to this genus.

REFERENCES

1. American Type Culture Collection. 1992. American Type Culture Collection catalogue of bacteria and bacteriophages, 18th ed. American Type Culture Collection, Rockville, Md.
2. Barksdale, L. 1981. The genus *Corynebacterium*, p. 1827–1837. In M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes, vol. 2. Springer-Verlag, New York.
3. Barreau, C., F. Bimet, M. Kiredjian, N. Rouillon, and C. Bizet. 1993. Comparative chemotaxonomic studies of mycolic acid-free coryneform bacteria of human origin. J. Clin. Microbiol. 31:2085–2090.
4. Briglia, M., R. I. L. Eggen, D. J. Van Es, and W. M. De Vos. 1994. Phylogenetic evidence for transfer of pentachlorophenol-mineralizing *Rhodococcus chlorophenolicus* PCP-1^T to the genus *Mycobacterium*. Int. J. Syst. Bacteriol. 44:494–498.
5. Bruce, D. L., J. E. Bismar, and J. M. Vickerstaff. 1969. Comparative examinations of virulent *Corynebacterium kutscheri* and its presumed avirulent variant. Can. J. Microbiol. 15:817–818.
6. Chun, J., and M. Goodfellow. 1995. A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. Int. J. Syst. Bacteriol. 45:240–245.
7. Collins, M. D., R. A. Burton, and D. Jones. 1988. *Corynebacterium amycolatum* sp. nov., a new mycolic acid-less *Corynebacterium* species from human skin. FEMS Microbiol. Lett. 49:349–352.
8. Collins, M. D., and C. S. Cummins. 1986. Genus *Corynebacterium* Lehman and Neumann 1896, p. 1266–1276. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams and Wilkins Co., Baltimore.
9. Collins, M. D., M. Goodfellow, and D. E. Minnikin. 1982. Fatty acid composition of some mycolic acid-containing coryneform bacteria. J. Gen. Microbiol. 128:2503–2509.
10. Collins, M. D., J. Smida, M. Dorsch, and E. Stackebrandt. 1988. *Tsukamurella* gen. nov. harboring *Corynebacterium paurometabolum* and *Rhodococcus aurantiacus*. Int. J. Syst. Bacteriol. 38:385–391.
11. Coyle, M. B., R. B. Leonard, D. J. Nowowiejski, A. Malekni, and D. J. Finn. 1993. Evidence of multiple taxa within commercially available reference strains of *Corynebacterium xerosis*. J. Clin. Microbiol. 31:1788–1793.
12. Cummins, C. S., and H. Harris. 1956. The chemical composition of the cell wall in some Gram-positive bacteria and its possible value as a taxonomic character. J. Gen. Microbiol. 14:583–600.
13. Fox, G. E., and E. Stackebrandt. 1987. The application of 16S rRNA cataloging and 5S rRNA sequencing in bacterial systematics. Methods Microbiol. 19:405–458.
14. Funke, G., S. Stubbs, M. Altwegg, A. Carlotti, and M. D. Collins. 1994. *Turicella otitidis* gen. nov., sp. nov., a coryneform bacterium isolated from patients with otitis media. Int. J. Syst. Bacteriol. 44:270–273.
15. Goodfellow, M. 1992. The family *Nocardiaceae*, p. 1188–1213. In A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), The prokaryotes, 2nd ed., vol. 2. Springer-Verlag, New York.
16. Goodfellow, M., A. R. Beckham, and M. D. Barton. 1982. Numerical classification of *Rhodococcus equi* and related actinomycetes. J. Appl. Bacteriol. 53:199–207.
17. Goodfellow, M., J. Chun, S. Stubbs, and A. S. Tobili. 1994. Transfer of *Nocardia amarae* Lechevalier and Lechevalier 1974 to the genus *Gordona* as *Gordona amarae* comb. nov. Lett. Appl. Microbiol. 19:401–405.
18. Goodfellow, M., J. Zakrzewska-Czerwinska, E. G. Thomas, M. Mordarski, A. C. Ward, and A. L. James. 1991. Polyphasic taxonomic study of the genera *Gordona* and *Tsukamurella* including the description of *Tsukamurella wratislaviensis* sp. nov. Zentralbl. Bakteriell. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. 275:162–178.
19. Groman, N., J. Schiller, and J. Russell. 1984. *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* responses to DNA probes derived from coryneform β and *Corynebacterium diphtheriae*. Infect. Immun. 45:511–517.
20. Häggblom, M. M., L. J. Nohynek, N. J. Palleroni, K. Kronqvist, E.-L. Nurmiho-Lassila, M. S. Salkinoja-Salonen, S. Klatte, and R. M. Kroppenstedt. 1994. Transfer of polychlorophenol-degrading *Rhodococcus chlorophenolicus* (Apalahti et al. 1986) to the genus *Mycobacterium chlorophenolicum* comb. nov. Int. J. Syst. Bacteriol. 44:485–493.
21. Huelsenbeck, J. P., and D. M. Hillis. 1993. Success of phylogenetic methods in the four-taxon case. Syst. Biol. 42:247–264.
22. Kämpfer, P., W. Dott, and R. M. Kroppenstedt. 1990. Numerical classification and identification of some nocardioform bacteria. J. Gen. Appl. Microbiol. 36:309–331.
23. Kim, J. 1993. Improving the accuracy of phylogenetic estimation by combin-

- ing different methods. *Syst. Biol.* **42**:331–340.
24. Klatte, S., K.-D. Jahnke, R. M. Kroppenstedt, F. Rainey, and E. Stackebrandt. 1994. *Rhodococcus luteus* is a later subjective synonym of *Rhodococcus fascians*. *Int. J. Syst. Bacteriol.* **44**:627–630.
 25. Klatte, S., F. A. Rainey, and M. Kroppenstedt. 1994. Transfer of *Rhodococcus aichiensis* Tsukamura 1982 and *Nocardia amarae* Lechevalier and Lechevalier 1974 to the genus *Gordona* as *Gordona aichiensis* comb. nov. and *Gordona amarae* comb. nov. *Int. J. Syst. Bacteriol.* **44**:769–773.
 26. Lechevalier, M. P., and H. Lechevalier. 1970. Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int. J. Syst. Bacteriol.* **20**:435–443.
 27. Liebl, W., M. Ehrmann, W. Ludwig, and K.-H. Schleifer. 1991. Transfer of *Brevibacterium divaricatum* DSM 20297^T, "*Brevibacterium flavum*" DSM 20411, "*Brevibacterium lactofermentum*" DSM 20412 and DSM 1412, and *Corynebacterium lilium* DSM 20137^T to *Corynebacterium glutamicum* and their distinction by rRNA gene restriction patterns. *Int. J. Syst. Bacteriol.* **41**:255–260.
 28. Mordarski, M. M., M. Goodfellow, A. Tkacz, G. Pulverer, and K. P. Schaal. 1980. Ribosomal ribonucleic acid similarities in the classification of *Rhodococcus* and related taxa. *J. Gen. Microbiol.* **118**:313–319.
 29. Pitcher, D. G. 1983. Deoxyribonucleic acid base composition of *Corynebacterium diphtheriae* and other corynebacteria with cell wall chemotype IV. *FEMS Microbiol. Lett.* **16**:291–295.
 30. Pitulle, C., M. Dorsch, J. Kadzda, J. Wolters, and E. Stackebrandt. 1992. Phylogeny of rapidly growing members of the genus *Mycobacterium*. *Int. J. Syst. Bacteriol.* **42**:337–343.
 31. Rainey, F. A., J. Burghardt, R. M. Kroppenstedt, S. Klatte, and E. Stackebrandt. 1995. Phylogenetic analysis of the genera *Rhodococcus* and *Nocardia* and evidence for the evolutionary origin of the genus *Nocardia* from within the radiation of *Rhodococcus* species. *Microbiology* **141**:523–528.
 32. Rainey, F. A., J. Burghardt, R. M. Kroppenstedt, S. Klatte, and E. Stackebrandt. 1995. Polyphasic evidence for the transfer of *Rhodococcus roseus* to *Rhodococcus rhodochrous*. *Int. J. Syst. Bacteriol.* **45**:101–103.
 33. Rainey, F. A., S. Klatte, R. M. Kroppenstedt, and E. Stackebrandt. 1995. *Dietzia*, a new genus including *Dietzia maris* comb. nov., formerly *Rhodococcus maris*. *Int. J. Syst. Bacteriol.* **45**:32–36.
 34. Riegel, P., D. de Briel, G. Prévost, F. Jehl, and H. Monteil. 1993. Proposal of *Corynebacterium propinquum* sp. nov. for *Corynebacterium* group ANF-3 strains. *FEMS Microbiol. Lett.* **113**:229–234.
 35. Riegel, P., D. de Briel, G. Prévost, F. Jehl, H. Monteil, and R. Minck. 1993. Taxonomic study of *Corynebacterium* group ANF-1 strains: proposal of *Corynebacterium afermentans* sp. nov. containing the subspecies *C. afermentans* subsp. *afermentans* subsp. nov. and *C. afermentans* subsp. *lipophilum* subsp. nov. *Int. J. Syst. Bacteriol.* **43**:287–292.
 36. Riegel, P., M. V. Kamne-Fotso, D. De Briel, G. Prévost, F. Jehl, Y. Piémont, and H. Monteil. 1994. *Rhodococcus chubuensis* Tsukamura 1982 is a later subjective synonym of *Gordona sputi* (Tsukamura 1978) Stackebrandt 1989 comb. nov. *Int. J. Syst. Bacteriol.* **44**:764–768.
 37. Riegel, P., R. Ruimy, R. Christen, and H. Monteil. Unpublished data.
 38. Riegel, P., R. Ruimy, D. De Briel, G. Prévost, F. Jehl, R. Christen, and H. Monteil. 1995. Genomic diversity and phylogenetic relationships among lipid-requiring diphtheroids from humans and characterization of *Corynebacterium macginleyi* sp. nov. *Int. J. Syst. Bacteriol.* **45**:128–133.
 39. Riegel, P., R. Ruimy, D. De Briel, G. Prévost, F. Jehl, R. Christen, and H. Monteil. 1995. Taxonomy of *Corynebacterium diphtheriae* and related taxa with recognition of *Corynebacterium ulcerans* sp. nov. nom. rev. *FEMS Microbiol. Lett.* **126**:271–276.
 40. Rogall, T., J. Wolters, T. Flohr, and E. C. Böttger. 1990. Towards a phylogeny and definition of species at the molecular level within the genus *Mycobacterium*. *Int. J. Syst. Bacteriol.* **40**:323–330.
 41. Ruimy, R., P. Boiron, V. Boivin, and R. Christen. 1994. A phylogeny of the genus *Nocardia* deduced from the analysis of small-subunit ribosomal DNA sequences, including transfer of *Nocardia amarae* to the genus *Gordona* as *Gordona amarae* comb. nov. *FEMS Microbiol. Lett.* **123**:261–268.
 42. Ruimy, R., V. Breittmayer, P. Elbaze, B. Lafay, O. Boussebart, M. Gauthier, and R. Christen. 1994. A general phylogenetic analysis of the family *Vibrionaceae* (*Vibrio*, *Photobacterium*, *Aeromonas*, *Plesiomonas*) deduced from the comparison of nearly complete 16S rRNA sequences. *Int. J. Syst. Bacteriol.* **44**:416–426.
 43. Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**:406–425.
 44. Smith, A. B., B. Lafay, and R. Christen. 1992. Comparative variation of morphological and molecular evolution through geologic time: 28S ribosomal RNA versus morphology in echinoids. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **338**:365–382.
 45. Sneath, P. H. A. 1993. Evidence from *Aeromonas* for genetic crossing-over in ribosomal sequences. *Int. J. Syst. Bacteriol.* **43**:626–629.
 46. Sritharan, V., and R. H. Barker. 1991. A simple method for diagnosing *Mycobacterium tuberculosis* infection in clinical samples using PCR. *Mol. Cell. Probes* **5**:385–395.
 47. Stackebrandt, E., W. Ludwig, E. Seewaldt, and K.-H. Schleifer. 1983. Phylogeny of sporeforming members of the order *Actinomycetales*. *Int. J. Syst. Bacteriol.* **33**:173–180.
 48. Stackebrandt, E., and C. R. Woese. 1981. Towards a phylogeny of the actinomycetes and related organisms. *Curr. Microbiol.* **5**:197–202.
 49. Stahl, D. A., and J. W. Urbance. 1990. The division between fast- and slow-growing species corresponds to natural relationships among the mycobacteria. *J. Bacteriol.* **172**:116–124.
 50. Suzuki, K., T. Kanebo, and K. Komagata. 1981. Deoxyribonucleic acid homologies among coryneform bacteria. *Int. J. Syst. Bacteriol.* **31**:131–138.
 51. Swofford, D. 1990. PAUP: phylogenetic analysis using parsimony, version 3.0. Illinois Natural History Survey, Champaign.
 52. Warwick, S., T. Bowen, H. McVeigh, and M. Embley. 1994. A phylogenetic analysis of the family *Pseudonocardiaceae* and the genera *Actinokineospora* and *Saccharothrix* with 16S rRNA sequences and a proposal to combine the genera *Amycolata* and *Pseudonocardia* in an emended genus *Pseudonocardia*. *Int. J. Syst. Bacteriol.* **44**:293–299.
 53. Yanagawa, R., and E. Honda. 1978. *Corynebacterium pilosum* and *Corynebacterium cystitidis*, two new species from cows. *Int. J. Syst. Bacteriol.* **28**:209–216.