Phylogeny of Sporeforming Members of the Order *Actinomycetales*

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Representatives from various genera of the order *Actinomycetales* were investigated by comparative cataloging of their 16S ribosomal ribonucleic acids to establish their phylogenetic relationships. Members of *Actinoplanes*, *Ampullariella*, *Dactylosporangium*, and *Micromonospora* form one cluster that is peripherally related to the cluster defined by members of *Mycobacterium*, *Nocardia*, and *Rhodococcus*. *Streptomyces griseus* and strains of *Chainia*, *Kitasatoa*, *Streptovercillium*, *Microellobosporia*, and *Elytrosporangium* are phylogenetically closely related, whereas *Streptosporangium roseum* is more distantly related to this cluster. *Thermomonospora curvata* and *Geodermatophilus obscurus* represent individual clusters. *Promicromonospora citrea* is a member of the *Arthrobacter-Micrococcus-Cellulomonas* line of descent. This grouping of organisms is highly supported not only by data from deoxyribonucleic acid-deoxyribonucleic acid homology studies and deoxyribonucleic acid-ribosomal ribonucleic acid cistron similarity studies but also by several common biochemical characteristics of proven taxonomic value.

In the first part of our investigation of the molecular genetic relatedness of sporeforming members of the order *Actinomycetales* (19), which was based on nucleic acid reassociation studies, we concluded that most of these organisms can be grouped into three major clusters. The first of these clusters includes members of *Actinoplanes*, *Ampullariella*, *Amorphosporangium*, *Dactylosporangium*, and *Micromonospora*, the second cluster contains *Streptosporangium roseum*, *Planobispora longispora*, and *Planomonospora parontospora*, and the third cluster contains representatives of various genera of the *Streptomycetaceae* (i.e., *Streptomyces*, *Kitasatoa*, *Chainia*, *Streptovercillium*, *Elytrosporangium*, and *Actinosporangium*).

Deoxyribonucleic acid (DNA)-DNA reassociation studies have shown that strains of each of these clusters are closely to moderately related. DNA-ribosomal ribonucleic acid (rRNA) cistron similarity experiments have supported this finding, and, in addition, the data obtained did not reveal any specifically closer relationship between any two of the three clusters.

The most successful method for unraveling the phylogenetic relatedness of higher taxa is comparative analysis of the ribonuclease T₁-resistant oligonucleotides of 16S rRNAs (4, 5, 18). More than 250 species of bacteria have already been investigated by this method, and the resulting phylogenetic tree is an impressive demonstration of its usefulness (5, 18). Analysis of 16S rRNAs allows workers to determine the

degree of relatedness of distantly related organisms and also provides a key to phylogenetic classification of bacteria (1).

Only a few representatives of the order *Actinomycetales* have been investigated with this method previously (5, 16, 17). Extending these studies, we not only included species of the homology clusters defined previously (19), but also determined the degree of relatedness between these organisms and strains of *Geodermatophilus obscurus*, *Nocardia asteroides*, *Thermomonospora curvata*, *Promicromonospora citrea*, and coryneform bacteria.

MATERIALS AND METHODS

All of the organisms investigated in this study are listed in Table 1. Information concerning the sources of these strains is also presented in Table 1. Except for *Thermomonospora curvata* DSM 43183^T (type strain), all organisms were grown aerobically at 30°C in 300 ml of yeast extract-peptone-sucrose broth as described previously (19). The medium for *Thermomonospora curvata* DSM 43183^T was prepared as follows. A 20-g portion of rolled oats was boiled for 20 min and filtered through Whatman no. 1 filter paper. The resulting filtrate was made up to 1,000 ml, 1 ml of a trace element solution was added (FeSO₄ · 7 H₂O, 0.1 g; MnCl₂ · 4 H₂O, 0.1 g; ZnSO₄ · 7 H₂O, 0.1 g; distilled water, 100 ml), and the pH was adjusted to 7.2. *Thermomonospora curvata* DSM 43183^T was grown aerobically in 500 ml of this medium at 50°C. The purity of each culture was checked microscopically. Bacteria were harvested after 2 to 4 days of growth by filtration through Whatman no. 1 filter paper.

TABLE 1. Binary comparisons among the 16S rRNA catalogs (S_{AB} values)^a

Serial no.	Organism ^b	S_{AB} values compared with the following strains:							
		1	2	3	4	5	6	7	8
1	<i>Ampullariella regularis</i> DSM 43151 ^T	1.00							
2	<i>Actinoplanes philippinensis</i> DSM 43019 ^T	0.80	1.00						
3	<i>Dactylosporangium aurantiacum</i> NRRL B-8111	0.71	0.72	1.00					
4	<i>Micromonospora chalcone</i> NRRL B-2344	0.75	0.75	0.76	1.00				
5	<i>Streptomyces griseus</i> Ensign 1947	0.46	0.50	0.51	0.49	1.00			
6	<i>Kitasatoa kauaiensis</i> DSM 43360 ^T	0.46	0.50	0.53	0.48	0.87	1.00		
7	<i>Chainia antibiotica</i> DSM 43032 ^T	0.47	0.49	0.52	0.51	0.70	0.71	1.00	
8	<i>Streptoverticillium baldacii</i> DSM 40845 ^T	0.46	0.48	0.53	0.51	0.82	0.82	0.75	1.00
9	<i>Microellobosporia cinerea</i> DSM 43033 ^T	0.47	0.48	0.49	0.47	0.78	0.79	0.78	0.76
10	<i>Elytrosporangium brasiliense</i> DSM 43159 ^T	0.47	0.50	0.52	0.50	0.69	0.70	0.82	0.68
11	<i>Streptosporangium roseum</i> DSM 43021 ^T	0.53	0.54	0.55	0.51	0.64	0.66	0.64	0.63
12	<i>Thermomonospora curvata</i> DSM 43183 ^T	0.45	0.44	0.44	0.48	0.51	0.53	0.54	0.52
13	<i>Geodermatophilus obscurus</i> DSM 43160 ^T	0.50	0.49	0.51	0.50	0.52	0.56	0.53	0.52
14	<i>Arthrobacter globiformis</i> DSM 20124 ^T	0.53	0.58	0.56	0.52	0.57	0.59	0.59	0.55
15	<i>Oerskovia turbata</i> ATCC 25835 ^T	0.54	0.56	0.59	0.58	0.54	0.55	0.58	0.54
16	<i>Cellulomonas flavigena</i> DSM 20109 ^T	0.46	0.49	0.53	0.52	0.51	0.52	0.56	0.50
17	<i>Promicromonospora citrea</i> DSM 43110 ^T	0.51	0.52	0.55	0.54	0.59	0.62	0.60	0.58
18	<i>Corynebacterium diphtheriae</i> ATCC 11913	0.45	0.52	0.53	0.48	0.50	0.50	0.49	0.47
19	<i>Mycobacterium phlei</i> ATCC 11758 ^T	0.53	0.59	0.62	0.57	0.51	0.52	0.51	0.48
20	<i>Nocardia asteroides</i> DSM 43005	0.60	0.65	0.62	0.58	0.58	0.61	0.64	0.62
21	<i>Thermoactinomyces vulgaris</i> P12	0.24	0.26	0.25	0.31	0.31	0.32	0.36	0.31
22	<i>Bacillus subtilis</i> ATCC 15563	0.24	0.29	0.30	0.26	0.31	0.33	0.37	0.34
23	<i>Escherichia coli</i> B-236	0.25	0.29	0.28	0.28	0.24	0.24	0.29	0.25

^a When strains within groups were compared, the following DNA-DNA homology values were obtained: for the group that included strains 1 through 4, 18 to 28%; for the group that included strains 5 through 10, 28 to 40%; for the group that included strains 15 and 16, 20%. When comparisons were made between groups, the following DNA-DNA homology values were obtained (14, 19): the group that included strains 1 through 4 compared with the group that included strains 5 through 10, 6 to 12%; the group that included strains 1 through 4 compared with strain 11, 11 to 12%; the group that included strains 5 through 10 compared with strain 11, 10 to 13%. Homology values for strains 12 and 13 and for strains 17 through 23 were not determined.

^b DSM, Deutsche Sammlung von Mikroorganismen, Göttingen, Federal Republic of Germany; ATCC, American Type Culture Collection, Rockville, Md.; NRRL, National Regional Research Laboratory Culture Collection, Agricultural Research Service, Peoria, Ill. *Streptomyces griseus* Ensign 1947 was kindly provided by Jerald C. Ensign, University of Wisconsin, Madison.

We isolated 16S rRNAs by breaking cells in a French press and then subjecting the phenolized lysate to one-dimensional slab gel electrophoresis, as described previously (16). Digestion of the rRNA with ribonuclease T₁ (Calbiochem-Behring, La Jolla, Calif.), 3'-end dephosphorylation of the resulting oligonucleotides, 5' labeling with polynucleotide kinase (Boehringer, Mannheim, West Germany) and 1 mCi of γ -³²P-labeled adenosine triphosphate (Amersham, Braunschweig, West Germany), removal of excess γ -³²P-labeled adenosine triphosphate with Apyrase (Sigma, Munich, West Germany), fingerprinting of the oligonucleotides, and sequence determination were done by the methods of Stackebrandt et al. (16). Binary matching coefficients (S_{AB}) were calculated as described by Fox et al. (4).

RESULTS

Table 2 lists the oligonucleotide catalogs for the strains investigated in this study, together with the catalogs for sporeforming members of the order *Actinomycetales* that have been published previously (16, 17). The S_{AB} values for the strains, various other gram-positive eubacteria, and *Escherichia coli* and the resulting dendrogram of relationships are shown in Table 1 and Fig. 1, respectively. More detailed relationships within the clusters containing representatives of the genera *Corynebacterium* and *Nocardia* (17) and representatives of the genera *Arthrobacter*, *Cellulomonas*, *Microbacterium*,

TABLE 1—Continued

S_{AB} values compared with the following strains:														
9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1.00														
0.71	1.00													
0.65	0.59	1.00												
0.52	0.49	0.58	1.00											
0.54	0.52	0.57	0.56	1.00										
0.56	0.59	0.54	0.52	0.53	1.00									
0.53	0.57	0.56	0.47	0.52	0.67	1.00								
0.51	0.53	0.50	0.47	0.49	0.66	0.71	1.00							
0.59	0.57	0.59	0.50	0.58	0.63	0.66	0.63	1.00						
0.48	0.48	0.47	0.40	0.43	0.57	0.53	0.50	0.54	1.00					
0.49	0.50	0.48	0.43	0.43	0.57	0.51	0.48	0.53	0.59	1.00				
0.59	0.56	0.59	0.49	0.41	0.56	0.55	0.49	0.62	0.57	0.69	1.00			
0.36	0.32	0.34	0.35	0.33	0.32	0.30	0.29	0.34	0.28	0.26	0.30	1.00		
0.35	0.34	0.35	0.33	0.33	0.32	0.31	0.29	0.36	0.31	0.29	0.34	0.52	1.00	
0.24	0.26	0.23	0.21	0.21	0.24	0.20	0.23	0.22	0.26	0.28	0.30	0.23	0.26	1.00

and *Brevibacterium* (15) have been published previously.

The sporeforming members of the order *Actinomycetales* investigated occurred in four lines of descent within the major subgroup of gram-positive bacteria exhibiting high DNA guanine-plus-cytosine contents (≤ 55 mol%). These lines were separated from each other by S_{AB} values of about 0.52 (Fig. 1).

Promicromonospora citrea DSM 43110^T, a highly pleomorphic organism, was found to be a member of a fifth line, which contained arthrobacters, cellulomonads, and oerskovias, as well as brevibacteria, micrococci, and microbacteria and their relatives (data not shown). Members of this line are related to strains in the other four lines of descent at an average S_{AB} value of 0.52.

DISCUSSION

The close phylogenetic relationship which we found among certain species of different genera (e.g., those in the *Streptomyces griseus* subcluster and those in the *Actinoplanes philippinensis* cluster) is in accord with data obtained from DNA-DNA reassociation studies (Table 1) and

with results of DNA-rRNA cistron similarity studies (19). Strains in these clusters share DNA homology values of 18% or greater, and the $\Delta T_{m(e)}$ values of the DNA-rRNA hybrids are 7°C or lower. On the other hand, the DNA homology values between representatives of the individual clusters are all 13% or less, whereas the $\Delta T_{m(e)}$ values are 10°C or higher.

The phylogenetic grouping of organisms reported here is strongly supported by common characters of proven taxonomic value (e.g., the chemical composition of the peptidoglycan, the sugar composition of the cell walls, and the lipid composition of the cells). All strains of the *Actinoplanes philippinensis* cluster exhibit peptidoglycan of the *meso*-diaminopimelic acid type (variation A1 γ according to Schleifer and Kandler [13]). A portion of the diaminopimelic acid residues are hydroxylated. In contrast to other representatives of the order *Actinomycetales*, alanine is replaced by glycine in position 1 of the peptide subunit of the peptidoglycan (7); this may be a valuable chemotaxonomic character. All members of the *Actinoplanes philippinensis* cluster contain xylose as a characteristic whole-cell sugar (8, 11). The cellular fatty acids

TABLE 2. Oligonucleotide catalogs^a

Type	Sequence ^b	Strain serial no.	Type	Sequence ^b	Strain serial no.
6-mers	CCCCCG	11, 13	6-mers	AUCCUG	1-13, 15, 17, 20
	CCCCAG	4, 15, 17		UCAAUG	13, 17
	CCCACG	12		UCACUG	17
	ACCCCG	1-4, 17, 20		UAACUG	7, 15, 17, 20
	CACACG	5-11		UAAUCG	1-13, 15, 17, 20
	ACACCG	6		AAUUCG	1, 2, 9
	CAAACG	15		AUAUCG	12
	CACAAG	1-7, 9-13, 15, 17, 20		AUACUG	1-4, 7, 10
	ACACAG	12		AAAUUG	13
	AAACCG	4, 12		AAUAUG	4
	AACCAG	9		UUUCCG	11
	AACAAG	10		UUCUCG	15
	AAAAAG	17		UCUUCG	2
	CCUCCG	6		UCUCUG	1-4, 15, 17, 20
	CCCUCG	12		UCCUUG	15
	CCCUG	11, 15		CCUUUG	12
	CCCUAG	3		ACUUUG	2
	CUCACG	17		AUAUUG	10
	UCCACG	1-13, 15, 20		UUUUCG	1
	CACCUG	17		UUUCUG	2
	CACUCG	15		CUUUUG	2
	CAUCCG	9	7-mers	CCCAAAG	11
	ACUCCG	15		AACACCG	1-7, 9-13, 15, 17
	CUAACG	1-13, 15, 17, 20		CAACCUG	4, 13
	UCACAG	4		CACAUCG	7
	UCAAAG	17		CAACUCG	1-13, 15, 17, 20
	UCAACG	8		CAUCACG	10
	CAACUG	15		UACACAG	17
	CACUAG	7, 10		ACUCACG	7, 9, 10
	CAUCAG	5		CACAAUG	1-10, 15, 17, 20
	AACCUG	13		UAACACG	1-13, 15, 17, 20
	ACACUG	13		ACACUCG	17
	ACAUCG	1, 9, 11		ACAUCG	8, 10
	AUACCG	1, 3, 4, 7-13, 15, 17, 20		UAACAAG	1-13, 15, 17, 20
	CUAAAG	3		UACAAAG	3, 6, 13, 15
	UAAACG	1-13, 15, 17, 20		AAACCUG	20
	AAACUG	7, 11, 12, 20		UUCCCCG	5, 6, 9
	ACAUAG	8		CCCUUCG	1-6, 8, 11, 12, 20
	AACUAG	5, 6, 9		CCCCUUG	13
	ACUAAG	15, 17		CUUCACG	1-4, 15, 17
	AAUACG	2-10, 12, 15, 17, 20		CACUUCG	2
	UUCCCG	1-13, 15, 17, 20		CAUCUCG	8
	CUUCCG	13		UCCAUG	17
	CCUUCG	1-13, 15, 17, 20		CUCAAUG	7
	CCCUUG	7, 10		UUCACAG	3
	UCUCAG	1-13, 15, 17, 20		CAAUCUG	5-10
	UUCCAG	1		UAACCUG	1-3, 11, 12, 15
	UCCAUG	15, 17		ACAUCUG	12
	CAUCUG	2, 7, 10		ACUCAUG	12, 13, 17
	CAUUCG	5, 6, 11		CAUUAAG	1-13, 15, 17, 20
	UACUCG	1-4		UAUACAG	5, 6, 8
	UACCUG	1-13, 15, 20			
	ACCUUG	4			
	ACUCUG	1, 7, 12			
	AUUCGG	12			

TABLE 2—Continued

Type	Sequence ^b	Strain serial no.	Type	Sequence ^b	Strain serial no.
	UACAAUG	4, 5, 7-10		AUUUAUCG	15
	UAAUACG	5-11, 13, 15, 17, 20		AAUUAUUG	5-13, 15, 17
	AUAUCAG	1-11, 13, 15, 17, 20		AUUUAUUG	1-4
	AUCAAUG	10		UUUUUUCG	2, 3, 11
	UAAAAAG	5			
	UCCUUCG	1-4	9-mers	CCACACCCG	12
	UUCCUG	4		CCCAACCG	9
	CCUUUCG	5, 8		CCAAACCCG	11
	UUCCAUG	13		CAACACCCG	1, 2, 4, 11
	AUCUCUG	5, 7-9		CCUAACCCG	1, 12
	AAUAUUG	1-9, 11, 13, 15, 17, 20		CAACCCUCG	1, 2, 4, 12, 13, 15
	UUUCCUG	1		CCUACCAAG	1-6, 8, 11-13, 15, 17, 20
	UUCUCUG	3, 11		CUCACCAAG	7, 9, 10
	UAUUAUG	15		CCAACUACG	1-4, 13, 20
	UUUUUCG	1, 4, 13, 17		UAACACCCG	3, 5-10, 15, 17, 20
8-mers	AAAACCCG	3, 13		UACACACCG	1-13, 15, 17, 20
	ACCCAAG	20		AUAACCCCG	4
	CCACACUG	1-13, 13, 15, 17, 20		CAACAACUG	13
	ACCCAUG	5-11, 13, 15		AAAACCCUG	10
	ACUCACAG	5, 8		AUACAACCG	7
	CAAAACUG	11-13		AUAACACCG	6
	CAAAACUG	4		CCCCCUUG	5, 6, 9
	AAAACCG	4, 12		CAACCCUUG	3, 5-11, 17, 20
	CCUCUCCG	1, 3, 4		ACUCCUACG	1-13, 15, 17, 20
	CCCCUUCG	12		CUAAUACCG	1-4, 8, 13
	CCCCUUG	7, 8, 20		CUAACUACG	5-12, 15, 17
	CCUACCG	12		ACUCAUAG	15
	CCUAUCAG	1-13, 15, 17, 20		CCCCUUAUG	1-13, 15, 17, 20
	CUCAAUCG	1, 3		CCUUACCG	11
	CUCAACUG	3		CCAUUACUG	5, 6, 8, 9
	CUUAACCG	12		CUAAACUUG	15
	CUUCACAG	20		CUUAAUUCG	5-13
	UCAACUG	1-13, 15, 17, 20		AUUAUUCG	1-4, 15, 17, 20
	CAUUCACG	6-9		UUCUCUUG	17
	CAUCUACG	13			
	ACACUUCG	2			
	ACUCAUCG	1, 2, 4			
	AAUUCGG	12			
	ACUCACUG	6	10-mers	CCUAACCCCG	1
	AUACCCUG	2-13, 15, 17, 20		ACAUCACCCG	12
	CUACAAUG	1-13, 15, 17, 20		ACAUCACCG	7, 10, 15, 20
	AAAUCUCG	1, 13		AAACACUCAG	11
	AAAACUUG	1, 2, 20		ACAACUCAAG	15
	UCCCUUCG	12		CUCAACUCCG	13
	UUCCACUG	17		CUUAACCCCG	6, 8, 10
	CCUCUUCG	11		CCAUCCACG	13
	CCCCUUG	8		ACCCUAUCCG	2
	UCAUCAUG	3		ACCUCCACG	11
	UCAUCAUG	1, 2, 4-11, 20		ACAUCCACG	5, 6, 8, 9
	ACUCAUUG	11		AAUACCCUG	3
	AAUUCCG	1-11, 13, 15, 17, 20		AUAACCCUG	1, 2
	AAUACUG	20		AACCACUUG	7, 10
	CUUUUACG	17		ACACUACAG	15
				ACAUAUACCG	5, 6, 8
				ACUCCUAAUG	20
				CUUAACACUG	12

TABLE 2—Continued

Type	Sequence ^b	Strain serial no.	Type	Sequence ^b	Strain serial no.
	UCUAAUACCG	5-12, 17, 20		AAUCUCAAAAAG	5-10
	CUCAACUUAG	17		CCUAACCCCUUG	2, 20
	UUCUUACACG	12		CCCCAAACUUUG	1
	UCUAAUACUG	15		AACCUUACCUAG	13
				AAUCCCUUAAAG	20
11-mers	CUCAACCCCAG	4		CCCUUCACUUAG	17
	CCCAACCCUUG	3, 4, 7, 8, 15		CCCUUCACUCUG	5, 6, 8
	AAAUCUCACAG	2		CAACUCUCUUCG	9
	AUAACUCCAAG	13		AAUAAUACUUAG	1
	CUUCACACAUG	20			
	CUCAUUCCACG	15	13-mers	CUAAAACUCAAG	1-13, 15, 17, 20
	ACCACUUACUG	11		UCAAUAUCAUG	13, 15, 17
	AACCUUACCUG	1-4, 20		UAAACCUCUUUCG	3
	CUUAACACAUG	1-13, 15, 17, 20		AUAUUCAUUCUCG	3
				UUAAAUAUUUUUG	12
	CUUAACUCCAG	11			
	AUAACACUCUG	5	14-mers	UAAACCUCUUUCAG	1, 2, 5-11, 13, 15, 17, 20
12-mers	CUCAACCCCAAG	1, 2, 20			
	AAUCCCAAAAAG	1-4, 11, 13, 15, 17	15-mers	AUACAACCACUACAG	10
	CCCUCCACUCCG	9			
	CCCAACCCCUUG	5, 6, 10, 13, 17	3' end	AUCACCUCUUUCU _{OH}	1-13, 15, 17, 20
	CAACAUUACACG	7, 10			
	AACCUUACCAAG	5-12, 15, 17			
	AAUCCCUAAAAG	12			

^a See Table 1 for strain serial numbers. The oligonucleotide catalogs of strains 3 through 5 have been published previously (17).

^b The asterisks indicate modified nucleotides.

of these strains include iso and anteiso branched acids together with large amounts of unsaturated fatty acids (8), and the major menaquinone of these organisms is of the MK9 (H4) type (9).

All members of the *Streptomyces griseus* subcluster contain LL-diaminopimelic acid and glycine in their cell walls, but unlike members of the *Actinoplanes philippinensis* cluster, glycine is involved in the cross-linkage of the peptide subunits, with alanine in position 1 (peptidoglycan variation A3γ according to Schleifer and Kandler [13]). No member of this cluster contains sugars of diagnostic value (11). The cellular fatty acids of these strains are composed only of iso and anteiso branched acids, and their menaquinones are predominantly of the MK9 (H8) and MK9 (H6) types (9). The close relatedness which we found among species from different genera of the family *Streptomycetaceae* is further corroborated by the results of phage typing experiments; polyvalent *Streptomyces* phages attack strains of the genera *Streptomyces*, *Chainia*, *Kitasatoa*, *Streptoverticillium*, *Elytrosporangium*, and *Microellobosporia* (12). *Planobispora longispora* DSM 43041^T and *Planomon-*

ospora parontospora DSM 43177^T are related to *Streptosporangium roseum* DSM 43021^T by DNA reassociation values of 38 and 33%, respectively (19). Members of this subcluster share a peptidoglycan of the A1γ variation (13). The peptidoglycan of these strains does not contain glycine (13), madurose is the whole-cell sugar (11), and all members of this subcluster synthesize 10-methyl branched and iso branched cellular fatty acids (8).

Thermomonospora curvata and *Geodermatophilus obscurus* show a low relationship to each other and are distinct from all other members of the order *Actinomycetales*. *Thermomonospora curvata* is not as closely related to *Thermoactinomyces vulgaris* (Fig. 1) as current taxonomy (3, 6) may indicate. The previous report of a close relationship between *Geodermatophilus obscurus* and *Mycobacterium phlei*, with an *S*_{AB} value of 0.78 (17), is in error; although it has not been definitely determined, the data reveal that the strain used in the previous study, *Geodermatophilus obscurus* Ishiguro 22-28, was mislabeled and cannot be regarded as a strain of *Geodermatophilus obscurus*.

FIG. 1. Dendrogram of relationships among sporeforming and nonsporeforming members of the order Actinomycetales, coryneform bacteria, *Bacillus subtilis*, and *Escherichia coli*. This figure was constructed by average linkage clustering (between the merged groups) from the S_{AB} values given in Table 1 and those published previously (15, 17). *Nocardia calcarea* is identical to *Rhodococcus erythropolis* (M. Goodfellow, personal communication).

Nocardia asteroides DSM 43005¹ is a member of the *Mycobacterium-Nocardia-Rhodococcus*-line of descent (17) (Fig. 1) and shows high S_{AB} values when it is compared with *Nocardia coralina*, *Nocardia calcarea*, *Brevibacterium ketoglutamicum*, and *Corynebacterium fascians* (S_{AB} , 0.73 to 0.78 [unpublished data]); the S_{AB} value is slightly lower (S_{AB} , 0.69) when this strain is compared with *Mycobacterium phlei*.

The placement of *Promicromonospora citrea* in the enlarged *Arthrobacter globiformis* cluster (Fig. 1) is not surprising. Strains of this species and of cellulomonads and oerskovias are known to have similar phospholipids (10) and isoprenoid quinones (2). Indeed, the S_{AB} values indicate a closer degree of relatedness of *Promicromonospora citrea* to strains of *Cellulomonas*, *Oerskovia*, *Arthrobacter*, and *Micrococcus* than to members of other genera in this cluster (e.g., *Mycobacterium*, *Brevibacterium*, and *Actinomyces*) (unpublished data). The peptidoglycan of *Promicromonospora citrea* contains alanine, glutamic acid, and lysine at a ratio of 4:1:1, which is similar to the ratio found in arthrobacters and micrococci (13).

The phylogenetic structure of the order Actinomycetales is not always in accord with the classification of these organisms (3, 6). This is not surprising since Actinomycetales classification has been based almost solely on morpholog-

ical characters and has been performed mainly for identification. Thus, the rather broad groups of organisms that are revealed by 16S rRNA analyses indicate the broad taxonomic value of several chemotaxonomic characteristics, including cell wall components, cellular fatty acid composition, and cellular sugars.

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